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Youdim et al.
Clin. Ecology
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Youdim et al.
Clin. Ecology
vol 7
p 62-66
1990

Youdim et al.
Clin. Ecology
vol 7
p 67-72
1990

Rea
Annual Meeting-American Academy of Environmental Medicine (I think this is a book of abstracts published by American academy of Environmental Medicine)
Sept 1995
p213-222

0735-9306

NLM 2/19

Griffiths et al.
Cytobios
vol 93
p49-66
1998

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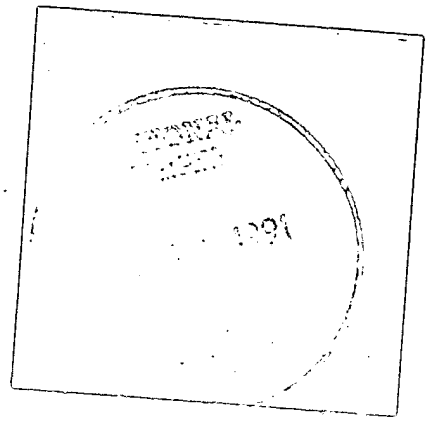

TREATMENT OF ENVIRONMENTALLY SENSITIVE PATIENTS WITH TRANSFER FACTOR

PART III: CASE STUDIES ON THREE PATIENTS

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ABSTRACT

Three case studies of environmentally sensitive patients treated with transfer factor (TF) are presented in this paper. Case 1 is a 44-year-old female with multiple allergies, hyper IgE and severe dermatitis. Case 2 is a 51-year-old female with multiple food and inhalant allergies and increased levels of blood chemicals. Case 3 is a 46-year-old female also with multiple allergies and environmental sensitivities. Upon treatment with TF, Case 1 demonstrated a most dramatic response with clearance of her eczematous skin, decrease in her serum IgE level, improved delayed cutaneous hypersensitivity (cellular immunity) and increase in the number of her suppressor cells. Case 2 demonstrated rapid clinical progress associated with improved immunologic parameters and decreased levels of toxic blood chemicals. Case 3, a highly reactive individual, showed slow but steady progress clinically and immunologically with increase in the number of lymphocytes, T cells, T cell subsets and CMI. This study indicated the diversity of patient population and response to TF therapy. *Transfer factor, T & B lymphocytes, cell mediated immunity, eczema, phenotype.*

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INTRODUCTION

In previous communications (1,2), we reported on the immunological and clinical response of a group of patients placed on a course of transfer factor (TF) therapy for a period of 6-12 months. The data presented the overall picture of our findings and the accumulated results. In this communication we present three representative case reports in order to familiarize the reader with specific examples of the patient population and their response to TF.

MATERIALS AND METHODS

Technical data patient selections, questionnaires, TF preparation and doses were as described previously (1,2). Immunoglobulin E determinations were performed using the Kallestad Quanti Clone kits (Kallestad Laboratories, Austin, Texas). Chemistries were performed by local laboratories. Blood toxic chemical levels were performed by AccuChem Laboratories (Richardson, Texas). Sensitivity to foods, molds, chemicals and hormones were determined by inhalation or intradermal challenge (3,4,5,6).

CASE SUMMARIES AND RESULTS

Case #1

A 44-year-old white female was admitted to the Environmental Control Unit with the chief complaint of urticaria. The patient reported that she had eczema and asthma as a child, and was hospitalized at the age of 1 for eczema. In mid-1978, she married and developed a severe erythematous rash with spreading of her eczema to her face, neck, arms, chest, and thighs. Burning, itching "sores" were a major problem. She noted that when she returned to her home in New Orleans there was a foam pad on her

mattress and upon exposure she had extreme whole-body dermatitis. She reported that certain foods and exposure to detergents and several other products caused the skin condition to worsen. She also had several flares of generalized urticaria from foods. She had tried many forms of therapy, but had only responded to steroids.

Her past medical history included a tonsillectomy and adenoidectomy in 1948, a benign breast tumor was excised in 1969. She had had several skiing accidents including bone fractures. There was a familial history of hay fever, cancer, eczema, depression, asthma, psychiatric care, heart disease, drug use, arthritis, drug allergies, and diabetes. Her mother died at the age of 54 of breast cancer. Her father was aged 70 with known salicylate problems, and sinus troubles. Two brothers both suffered from hay fever, and a sister had eczema.

Physical examination was normal except for the skin which showed scaly, erythematous rash over the upper trunk and extremities and upper legs. Rough, scaly skin all over the body was noted. Many areas were moist with exudate.

Intradermal tests showed positive reactions to 50 foods, molds, lake algae, T.O.E., Candida, house dust mite, smut, histamine, serotonin II, trees, weeds, terpenes, flagon, MRV, Nystatin, orris root, newsprint, perfume, formaldehyde and phenol.

Chemical challenges to assess her sensitivities were done in a steel and glass airtight booth by double blind method (3). Chemicals that produced symptoms were phenol (<.002ppm), an insecticide (<.034ppm), chlorine (<.33ppm), petroleum derived ethanol (<.5ppm) and normal saline.

She was asked to keep an environmentally clean oasis at home, eat less chemically contaminated foods and water, and take antigen injections for foods and inhalants. This resulted in some improvement, allowing the cessation of steroids. She found that heat made her more ill, so she moved from New Orleans to Chicago with benefit. The patient also found that with food immunotherapy her adverse foods reactions were controlled. She also noted that her symptoms were worse in the country, when exposed to plants, pollens and terpenes.

The patient was placed on 2 units of transfer factor per week on 10/23/86. This resulted in a diminution in her allergies, fatigue and an improvement in her ability to concentrate (see patient questionnaire refs. 1, 2). Her skin began to clear and her lymphocytes, total T cells, T helper and T suppressor cells increased significantly (Table 1). Her cell mediated immunity improved markedly (Table 2) with the positive response rising from one to four antigens and the sum of the mean reaction size growing from 2 to 26mm. At the same time there was a dramatic decrease in her IgE level (Table 1).

TABLE 1
Sequential WBC, T Cells Numbers and IgE Levels for Case 1

Immune Parameters	Before Treatment					After Treatment	Normal Range
	1981	1982	1983	1984	1985	1988	
WBC	6800	8700	5800	7000	7100	7500	4800-10000/mm^3
Lymphocyte(%)	0017	0021	0021	----	0014	0027	21-49
Total Lymphs	1156	1827	1218	1470	0994	2025	1400-4200/mm^3
T11 (%)	0073	0050	0065	0071	0082	0085	62-86
Total T11	0844	0914	0792	1044	0815	1721	1260-2560/mm^3
T4 (%)	----	----	----	0074	0060	0071	32-54
Total T4	----	----	----	1088	0596	1438	0670-1800/mm^3
T8 (%)	----	----	----	0008	0021	0012	17-35
Total T8	----	----	----	0118	0209	0243	0333-1070/mm^3
T4/T8	----	----	----	0009.25	0003.0	0005.9	01-2.7
B (%)	0022	----	0010	----	0013	0010	05-18
Total B	0254	----	0122	----	0129	0203	0082-0411/mm^3
Eosinophils (%)	0037	0011	0017	----	0012	0001	0-4%
Eos. Total	2182	0968	1197	----	----	----	0150-0250/mm3
IgE	1048	0610	>8000	----	18400	13156	10-15 IU/ml

TABLE 2
Increase in CMI After Transfer Factor Therapy, Case 1

Antigens Tested	Before Treatment 1985	After Treatment 1987
Tetanus	2	0
Diphtheria	0	7
Streptococcus	0	8
Tuberculin	0	5
Control	0	0
Candida	0	6
Trichophyton	0	0
Proteus	0	0
Scoring		
Number of positive reactions	1	4
Sum of average (mm)	2	26

Case #2

This 51-year-old white female was admitted with the chief complaints of urinary frequency and urgency, abdominal bloating, cramping, muscle and joint pain and headaches. She had had ongoing symptoms since childhood with numerous respiratory infections. Additionally during high school she developed chronic constipation which has persisted to date. Diagnostic evaluation by intradermal challenge showed the patient was sensitive to 22 foods, 4 weeds, 4 trees, 3 grasses, 6 chemicals, hormones, 7 molds, 2 smuts and histamine. The patient was treated with immunotherapy for her sensitivities. She also received supportive therapy with nutritional, vitamin and mineral supplementation.

She was placed on two units of transfer factor per week in 1987. Ten months after the start of TF therapy, she reported improvements in her allergic reactions, recurrent infections, fatigue, concentration, bowel problems and depression (see patient questionnaire 1, 2). Table 3 shows the patient's WBC and lymphocyte counts before and after seven months of TF therapy. There was a marked increase in her T cells and T cell sub populations. Her CMI response (Table 4) also improved from one barely positive reaction of 3mm to two reactions with a sum of 11mm in 1988 and to 6 reactions of 30mm in 1989. Her response to diphtheria antigen increased, and she developed de novo responses to strep, tuberculin, candida, trichophyton and proteus.

TABLE 3
WBC, Lymphocyte and T Cell Numbers for Case 2

Immune Parameters	Before Treatment 10/20/87	After Treatment 5/19/88	Normal Range
WBC	5200	4900	4800-10000/mm^3
Total lymphs	0728	1470	1400-4200
Lymphocyte %	0014	0030	0021-0049
T11 %	0089	0088	0062-0086
Total T11	0648	1294	1260-2650/mm^3
T4 %	0068	0058	0032-0054
Total T4	0495	0853	0670-1800/mm^3
T8 %	0014	0016	0017-0035
Total T8	0102	0235	0333-1070/mm^3
T4/T8	0004.8	0003.6	0001-0002.7
B %	0010	0003	0005-0018
Total B	0073	0044	0082-0477/mm^3

the patient's WBC and lymphocyte counts before and after seven months of TF therapy. There was a marked increase in her T cells and T cell sub populations. Her CMI response (Table 4) also improved from one barely positive reaction of 3mm to two reactions with a sum of 11mm in 1988 and to 6 reactions of 30mm in 1989. Her response to diphtheria antigen increased, and she developed de novo responses to strep, tuberculin, candida, trichophyton and proteus.

Case #3

This 46-year-old white female was admitted with the chief complaint of right frontal sinus headaches for the past 9 years. Other symptoms were recurrent nausea and vomiting, gas, abdominal bloating and occasional sore throat. Recurrent fatigue and lassitude was a large problem as well. Additionally, she had adrenal insufficiency and had also had several operations in 1975 and 1976. The

TABLE 4
Increase in CMI After Transfer Factor Therapy, Case 2

Antigens Tested	Diameter in Millimeters		
	Before Treatment	After Treatment	
	1987	1988	1989
Tetanus	0	0	0
Diphtheria	3	6	4
Streptococcus	0	0	4
Tuberculin	0	0	5
Control	0	0	0
Candida	0	0	5
Trichophyton	0	0	4
Proteus	0	5	6
Scoring			
Number of positive reactions	1	2	6
Sum of average (mm)	3	11	30

TABLE 5
WBC, Lymphocyte and T Cell Numbers for Case 3

Immune Parameters	Before Treatment		After Treatment	Normal Range
	9/2/85	4/1/86	5/6/87	
WBC	5100	4000	4600	4800-10000/mm^3
Lymphocytes %	0023	0030	0058	0021-0049
Total Lymphocytes	1173	1200	2669	1600-4200/mm^3
T11	0072	0080	0087	0062-0086
Total T11	0845	0960	2231	1260-2650/mm^3
T4 %	0054	0044	0048	0032-0054
Total T4	0633	0528	1281	0670-1800/mm^3
T8 %	0019	0034	0025	0017-0035
Total T8	0223	0408	0667	0333-1070/mm^3
T4/T8	0002.8	0001.3	0001.9	0001-0002.7
B Lymphocyte %	0005	0008	0005	0005-0018
Total B	0059	0096	0133	0082-0477/mm^3

following food challenges produced symptoms of headaches, fatigue, nausea and vomiting: pork, turkey, pineapple, rabbit, perch, broccoli, frog legs, duck, millet, moose, spinach, coconut, venison, catfish, goat milk, strawberries, barley, lima beans, dates, grapefruit, beef, eggs and zucchini.

Inhalation of phenol (0.4%) for less than 10 minutes produced yawning, sleepiness, nausea, confusion and fatigue. Natural gas exposure for 21 minutes caused tightness of sinuses, hands tingling, heart pounding, shortness of breath, dizziness, nausea and pressure headache. Inhalation of ethanol (50%) for less than 5 minutes triggered nasal stuffiness, sleepiness, yawning and postnasal discharge. Three sniffs of insecticide (Raid liquid ant and roach killer) caused nasal stuffiness, a cough, yawning and watery eyes. When exposed to formaldehyde for less than 10 minutes, she developed nasal congestion, itchy eyes, nausea, tight sinuses and sleepiness. Five minutes of challenge with chlorine (5.2%) caused nasal congestion, sore throat, watery eyes, and yawning.

The patient received antigen immunotherapy and was placed on two units of transfer factor per week in 1985. Progress was slow, but she reported a diminution in her allergic reactions, headache, recurrent infections and fatigue. (See patient questionnaire refs. 1, 2). Table 5 shows the patient's WBC and lymphocyte enumeration before and after TF therapy. Data gathered in 1987 showed a marked increase in her total lymphocyte count, total T cells and T cell populations. Table 6 shows the patient's CMI response in 1986 and 1987. Both these tests were obtained after initiation of TF therapy. CMI reactions prior to TF therapy were not available, nevertheless definite improvements in both the number and the size of the reactions was noted as therapy progressed.

TABLE 6
Increase in CMI During Transfer Factor Therapy
Case 3

Antigen Tested	Diameter (mm)	
	1986	1987
Tetanus	0	0.0
Diphtheria	4	0.5
Streptococcus	0	0.0
Tuberculin	9	5.5
Control	0	3.0
Candida	0	4.5
Trichophyton	0	3.0
Proteus	0	4.0
# of Positive Reactions	2.0	5.0
Sum of Average (mm)	13	25

DISCUSSION

Patient M.S. (Case 1) is a 44 year old female with multiple allergies, hyper IgE and severe dermatitis. Despite years of immunotherapy, this patient made modest progress until placed on TF therapy. The most dramatic response was the clearing of her eczema, the decrease in her IgE level and the increase in the number of her suppressor cells. An association between numerical deficiency of immunoregulatory T suppressor cells and atopic eczema has been reported (7,8). Abnormalities in polymorphonuclear and mononuclear cell chemotaxis and in cell mediated immunity have also been described in hyper IgE syndromes (9). Jones, et. al. (10) reported the use of oral bovine TF (OTF) in a child with skin eruptions due to hypersensitivity to eggs and milk with repeated skin infections, oral thrush and chronic cough. Administration of TF twice weekly for 6 months led to clinical improvements with associated laboratory data. These results closely parallel our findings and indicate possible usefulness of TF in these patients.

M.H. (Case 2) is a more typical patient in that she had been seen by numerous physicians including a neurologist, gynecologists, family practitioners, chiropractors and allergists without much improvement in her symptoms. She is also of interest as the improvement in her symptoms was rapid and was associated with a substantial decrease in the levels of the toluene, trichloroethylene and heptachlor epoxide in her blood. Although the association between TF therapy and a fall in the levels of toxic chemicals in the blood is not the purpose of this report, we have other data which supports this possibility. This patient also had a substantial increase in the number of her lymphocytes, T cells and T cell subsets.

Patient V.B. (Case 3) is a very sensitive patient with multiple allergies and sensitivities of long duration. Her progress was slow, but nevertheless dramatic, both clinically and as determined by laboratory data (Tables 5, 6). The number of her T cells as well as her CMI responses increased substantially during TF therapy. She was one of very few patients who reacted to the control glycerin on the CMI test (Table 6).

The above case summaries and data provided in the accompanying communications (1,2) represent comprehensive information gathered on a set of environmentally ill patients treated with TF. We feel these studies demonstrate the efficacy, safety and usefulness of TF in the treatment in certain environmentally ill patients. Similarly Bagnato and co-workers (11) reported that the degree of bronchial responsiveness to methacholine was significantly reduced at 90 days during treatment with thymomodulin and remained reduced, even if not significantly, 60 days after cessation of treatment.

Thymomodulin is a calf thymus acid lysate capable of improving the clinical symptoms of children with atopic dermatitis (12) and food allergy (13). Further, thymomodulin has been shown to regulate maturation of human and murine Pre-T-lymphocytes, and to modulate the function of mature human T & B lymphocytes for the regulation of immunoglobulin production (14,15). The effects of thymomodulin (16) and TF (1,2,17) might well be in modifying the entire immune response. It compares with the rather specific stimulation induced by allergen injections. It may potentiate the results of immunotherapy.

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(Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07717369 Genuine Article#: 200PB Number of References: 207
Title: Functional somatic syndromes
Author(s): Barsky AJ (REPRINT) ; Borus JF
Corporate Source: BRIGHAM & WOMENS HOSP,DIV PSYCHIAT, 75 FRANCIS ST/BOSTON//MA/02115 (REPRINT)
Journal: ANNALS OF INTERNAL MEDICINE, **1999**, V130, N11 (JUN 1), P 910-921
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Language: English Document Type: REVIEW
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Subfile: CC LIFE--Current Contents, Life Sciences; CC CLIN--Current Contents, Clinical Medicine;
Journal Subject Category: MEDICINE, GENERAL & INTERNAL
Abstract: The term functional somatic syndrome has been applied to several related syndromes characterized more by symptoms, suffering, and disability than by consistently demonstrable tissue abnormality. These syndromes include multiple **chemical sensitivity**, the sick building syndrome, repetition stress injury, the side effects of silicone breast implants, the Gulf War syndrome, chronic whiplash, the chronic fatigue syndrome, the irritable bowel syndrome, and fibromyalgia. Patients with functional somatic syndromes have explicit and highly elaborated self-diagnoses, and their symptoms are often refractory to reassurance, explanation, and standard treatment of symptoms. They share similar phenomenologies, high rates of co-occurrence, similar epidemiologic characteristics, and higher-than-expected prevalences of psychiatric comorbidity. Although discrete pathophysiologic causes may ultimately be found in some patients with functional somatic syndromes, the suffering of these patients is exacerbated by a self-perpetuating, self-validating cycle in which common, endemic, somatic symptoms are incorrectly attributed to serious abnormality, reinforcing the patient's belief that he or she has a serious disease. Four psychosocial factors propel this cycle of symptom amplification: the belief that one has a serious disease; the expectation that one's condition is likely to worsen; the ''sick role,'' including the effects of litigation and compensation; and the alarming portrayal of the condition as catastrophic and disabling. The climate surrounding functional somatic syndromes includes sensationalized media coverage, profound suspicion of medical expertise and physicians, the mobilization of parties with a vested self-interest in the status of functional somatic syndromes, litigation, and a clinical approach that overemphasizes the biomedical and ignores psychosocial factors. All of these influences exacerbate and perpetuate the somatic distress of patients with functional somatic syndromes, heighten their fears and pessimistic expectations, prolong their disability, and reinforce their sick role. A six-step strategy for helping patients with functional somatic syndromes is presented here.
Identifiers--KeyWord Plus(R): CHRONIC-FATIGUE-SYNDROME; IRRITABLE-BOWEL-SYNDROME; RANDOMIZED CONTROLLED TRIAL; TERM FOLLOW-UP; MULTIPLE CHEMICAL SENSITIVITIES; POSTTRAUMATIC-STRESS-DISORDER; COGNITIVE-BEHAVIORAL THERAPY; MASS PSYCHOGENIC ILLNESS; CARE-SEEKING BEHAVIOR; UPPER LIMB PAIN
Cited References:
*I MED, 1996, HLTH CONS SERV PERS

(Item 38 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03444499 BIOSIS NO.: 000023017587
CONTROLLED STUDIES ON FOOD SENSITIVITY USING INJECTION THERAPY

AUTHOR: **REA W J**; WILLIAMS M L; SPRAGUE D E
AUTHOR ADDRESS: DALLAS, TEXAS.

JOURNAL: 37TH ACA (AMERICAN COLLEGE OF ALLERGISTS) SCIENTIFIC CONGRESS, WASHINGTON, D.C., USA, APRIL 4-8, 1981. ANN ALLERGY 47 (2). 1981. 139.
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DESCRIPTORS: ABSTRACT HUMAN RECURRENT LARYNGEAL EDEMA
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- 16006 Respiratory System-Pathology
- 22018 Pharmacology-Immunological Processes and Allergy
- 34508 Immunology and Immunochemistry-Immunopathology, Tissue Immunology
- 35500 Allergy
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- 22502 Toxicology-Foods, Food Residues, Additives and Preservatives

BIOSYSTEMATIC CODES:
- 86215 Hominidae

BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
- Animals
- Chordates
- Vertebrates
- Mammals
- Primates
- Humans

4/9/80 (Item 2 from file: 65)
DIALOG(R)File 65:Inside Conferences 1993-1999/May W5

01474979 INSIDE CONFERENCE ITEM ID: CN014639864
The Potential Uses for Autogenous Lymphocyte Factors (ALF) in Environmental Medicine

Rea, W.

CONFERENCE: American Academy of Environmental Medicine: The cutting edge of environmental medicine-Annual meeting; 30th
ANNUAL MEETING- AMERICAN ACADEMY OF ENVIRONMENTAL MEDICINE, 1995; 30th
P: 213-222
The Academy, 1995
LANGUAGE: English DOCUMENT TYPE: Conference Abstrcts, presentations, papers and programme

CONFERENCE SPONSOR: American Academy of Environmental Medicine
CONFERENCE LOCATION: Tucson, AZ
CONFERENCE DATE: Sep 1995 (19950) (19950)

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The role of the T lymphocytic cell cycle and an autogenous lymphocytic factor in clinical medicine

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Abstract

In this study 315 individuals (25 controls, 290 chemically sensitive immunocompromised patients) were investigated. Each patient had been on a standard therapy of avoidance of pollutants, nutritional supplementation, and injections of antigens for foods, and biological inhalants, but did not attain their immunological competence. Peripheral lymphocytes were collected and DNA histograms were constructed. The flow cytometer was used to evaluate the cell cycle, haematological, and other immunological profiles. From the other portion of the blood specimen, lymphocytes were propagated *in vitro*, harvested, and a lysate, termed the autogenous lymphocytic factor (ALF), was prepared. When treated with ALF, 88% of these individuals showed a significant ($p < 0.001$) clinical improvement which correlated with laboratory findings, involving regulation of abnormal cell cycles, increase in total lymphocytes and subsets T_4, T_8, ($p < 0.05$) and cell mediated immunity (CMI) response ($p < 0.001$). The ALF presumably acts as a biological response modifier. The cell cycle and ALF provide clinical tools for diagnosis and regulation of immunological incompetence.

Introduction

The cell cycle is the ordered and orderly events of biochemical and morphological sequences, leading from the formation of a daughter cell as a result of mitosis, to the completion of the processes required for its own division into two daughter cells (Lee and Dang, 1995). It is fundamental that following mitosis, two daughter cells are produced. These cells may initiate a new cycle (G_1 phase), some cells may become nonproliferative (G_0 phase) or progress to a restriction point where they are committed to the synthesis of cellular components, (the S phase) and finally complete the cycle (the G_2M phase). The cell cycle then comprises the sum of the growth phases of a specific cell cycle. This cycle is repeated by continuously dividing cells. Even within the same organism the specific cell cycle will vary with different classes of cells. The total time that is necessary to complete the S and G_2 phases is generally constant in different cell types. It seems reasonable then, to assume that most of the time variation takes place in the G_1 phase.

To establish homeostasis, it is imperative that the cell cycle be regulated. To this end, there are biological parameters which may be employed to ascertain the normality of a particular cell cycle. The DNA content in the nucleus of a cell (2 N or diploid amount) is constant in all normal organisms (Givan, 1992). There are only two exceptions where the DNA content is not constant; the amount of DNA will vary in cells which undergo meiosis in preparation for sexual reproduction, thus containing the 1 N or haploid amount of DNA. The other exception applies to those cells which undergo DNA synthesis in preparation for mitosis. These will contain between 2 N and 4 N amounts of DNA.

By employing the concept of DNA constancy in a particular organism, and the application of flow cytometric techniques, DNA flow histograms can be constructed depicting a normal cell cycle and the identification of abnormal cycles.

The regulation of the cell cycle in eukaryotes seems to take place at two main transition points, prior to DNA replication at a point in the G_1 phase, termed the restriction point, and prior to cytokinesis at the G_2M phase boundary (Nurse and Bissett, 1981). The progression of the cell cycle from one phase to the other is mediated by specialized proteins, cyclins, and the activation of enzymes called cyclin-dependent kinases (CDKs) and by a number of positive and negative feedback loops. To date seven CDKs have been identified and designated CDK 2 to 8 (Meyerson *et al.*, 1992; Lorinez and Reed, 1984; Nurse, 1994; Sherr, 1994; Helchman and Roberts, 1994; King *et al.*, 1994; Hunter and Pines, 1994). Each CDK acts at different stages of the cell cycle and is differentially regulated by different cyclins (Meyerson and Harlow, 1994). The critical roles played by cyclins in the regulation of eukaryotic cell cycle is amply documented.

These CDKs play very significant roles in the G_1S and G_2M transitions during the mammalian cell cycle. They regulate by phosphorylation, a number of key substrates which subsequently activate a transition from G_1 to S and from G_2 to M (Kamp *et al.*, 1994). The catalytic subunits alone of these CDKs are not active and require the influence of positive regulatory subunits to ensure biochemically active protein kinase holoenzymes (Desai *et al.*, 1992). The positive regulatory subunits employed to this end are cyclins. The activity of CDKs is, therefore, regulated by both cyclins and specific phosphorylation and dephosphorylation (Gold and Nurse, 1989; Draetca and Beach, 1988; Solomon *et al.*, 1990; Gutier *et al.*, 1991; Murray and Krischner, 1989).

Cyclins were identified originally as proteins in the murine invertebrate cells. The concentration of these cyclins accumulate and are destroyed periodically at defined points during the cell cycle (Evans *et al.*, 1983). At present, eight cyclins have been identified; these are designated A, B, C, D, E, F, G_1, and H based on their amino acid sequences (Pines and Hunter, 1989; Xiong *et al.*, 1991), and in some instances, on genetic complementation experiments in yeast (Nurse, 1990; Murray and Krischner, 1989; Lewin, 1990; Sherr, 1993).

The influences of the cyclins are expressed differently; *e.g.* cyclin A exhibits its influence through the S-M phase (Wang *et al.*, 1990; Fang and Newport, 1991; Walker and Maller, 1991; Lehner and O'Farrell, 1990; Pagano *et al.*, 1992; Zindy *et al.*, 1992) of most cells, while cyclin B (which is conserved from yeasts to humans) propels cells into mitosis (Nurse and Bissett, 1981; Evans *et al.*, 1983; Glotzer *et al.*, 1991). Both the A and B cyclins are degraded at the M phase by ubiquitin (Ub)-dependent proteolysis (Glotzer *et al.*, 1991). G_1 cyclins (CIN 1, CIN 2, CIN 3) are assumed to associate with a p34 CDC2 homologue, p34 CDC 28, driving yeast cells into S phase (Reed, 1992; Cross, 1988; Richardson *et al.*, 1989; Hadwiger *et al.*, 1989; Wittenberg *et al.*, 1990; Nasmyth, 1990).

In the Environmental Health Center - Dallas (EHC-D), clinical cases are investigated varying from surgical to environmental illnesses. However, cases involving chemical sensitivity entail the principal aspect of our practice. Over a period of 22 years our laboratory observations show that characteristically, a percentage of the chemically sensitive individuals portray a secondary deficit which is related to immune dysfunction, principally, depression of the suppressor T-cell (Rea *et al.*, 1986, 1987).

Initial profiles of lymphocyte cell cycle progression, T and B lymphocytes, cell mediated immunity and haematograms of patients were compared with those taken subsequent to treatment with autogenous lymphocytic factor (ALF). Acting presumably as a biological response modifier, ALF was observed to modulate deregulated immune profiles.

The focus of this investigation was firstly to observe the patterns of DNA histograms of the T lymphocyte cell cycles, especially in chemically sensitive individuals. The histograms provide a 'snapshot' of the individual's present cell cycle. By comparing an individual's cell cycle with the classic normal cycle, very crucial information will be obtained to enable a scientific regulation of a patient's cell cycle. Secondly the work was to prepare a lysate

from the *in vitro* propagated peripheral T lymphocytes of an individual with an irregular T lymphocytic cell cycle, and observe the regulatory effect of this lysate, when injected subcutaneously into such an individual. Thirdly, the intention was to establish a basis for the regulation of an individual's T lymphocytes which were observed to be irregular due to varied incitants, thus restoring normal T lymphocyte functions, and the enabling of a compromised individual to cope with multiple insults to his/her immune system. Fourthly, we examined the responsiveness of T lymphocytes to autogenous lymphocytic factor (ALF) as measured by the cell mediated immunity (CMI) test, and to establish on an individual basis an index of T lymphocyte ability to respond.

From our search of databases, and to the best of our knowledge there is no documentation pertaining to the regulation of the T lymphocytic cell cycle by lysates of an individual's T lymphocytes, and its application to clinical medicine.

Materials and methods

Isolation of lymphocytes

Venous blood was collected from 315 individuals in heparinized tubes. Of these 25 were healthy employees of EHC-D; these represented normal controls. The other individuals were chemically sensitive, chronically ill patients of the EHC-D and referrals from outside physicians. Erythrocytes were separated by a modified gradient technique using heparinized CPT vacutainers (Becton Dickinson Co.). This preparation was centrifuged for 30 min at 500 x *g*. The lymphocyte layer (interphase between isolymph and plasma) was removed to sterile polystyrene tubes. Lymphocytic layers were washed three times with normal saline (0.9% sodium chloride) by centrifugation in a refrigerated centrifuge at 500 x *g* for 20 min. Cells were resuspended in normal saline and counted.

Lymphocyte phenotyping

Monoclonal antibodies against pan T cells, T_{11} (CD2), T helper cells T_4 (CD4), T suppressor/cytotoxic cells T_8 (CD8), activated T cells TA1 (CD26) and pan B cells B_4 (CD19) were bought from Coulter Immunology (Hialeah, Florida). Peripheral lymphocytes were obtained by venipuncture, and stained by the use of Coulter Q prep Epics immunology station following manufacturer's instructions.

Preparation of cell cycles

Heparinized whole blood or lymphocytes were used and 100 µl aliquots of heparinized whole blood or 100 µl of washed isolated lymphocytes (suspended in normal saline) were placed in 13 x 75 mm tubes. To each tube was added 200 µl of lysing buffer (Coulter) and gently mixed for ~15 s, then 2.0 ml of DNA stain with RNAse was added and mixed for 20 s The mixture was stained with propidium iodide at a concentration of 50 mg per ml, and allowed a staining period of 15 min and then analysed by flow cytometry.

Flow micro-fluorometric analysis

Cells tagged fluorometrically for DNA content were analysed on the Coulter Epic C flow cytometer, where they were allowed to pass through a laser beam tuned to 488 nm. Fluorescence was measured electronically and recorded as a histogram. DNA distribution in the cell cycle was calculated on accumulated data by parametric analysis to produce a particular DNA histogram.

Preparation of autogenous lymphocytic factor (ALF)

Propagation of lymphocytes

To cell culture flasks, containing 10 ml RPMI 1640 medium and/or supplemented with 1 ml bovine calf serum was added 1 ml of washed T lymphocytes. The culture was incubated at 37°C and monitored daily for approximately 2 to 3 weeks.

Harvesting of cells

When the cell population approximated 5 to 9 x 10^6 per ml, culture suspensions were transferred to calibrated 50 ml conical tubes and centrifuged for 30 min at 500 x *g*. The supernate was discarded, cell pellet resuspended in 10 ml normal saline and washed three times by centrifugation for 30 min each time.

Preparation of ALF

Each cell pellet was resuspended in ~2.0 ml normal saline and sonicated at 20 W with a duty cycle of 50% for 60 s. The sonicated mixture was sterilized by filtration and stored at –20°C to be diluted for therapeutic use as indicated.

Establishment of a functional T lymphocyte cell cycle

The profile of an individual's T lymphocyte cell cycle may appear normal, yet the lymphocytes may not be functional. A process was established to evaluate the immunological responsiveness of the lymphocytes of the cell cycle.

Stimulation of peripheral lymphocytes

Nunclon™-plastic plates (24 wells) containing 2 ml of RPMI 1640 medium per well were inoculated with ~2 to 4 x 10^6 lymphocytes per ml, 1.25 ng interleukin 1 alpha (Il_1 alpha) per ml, or with 0.1 ml ALF per ml; negative controls had neither Il_1 nor ALF. The plates were incubated at 37°C for 96 h. Cells were harvested, washed and analysed by flow cytometrically as mentioned above. Comparison of the S and G_2 phases of both the unstimulated and the stimulated T lymphocytes was undertaken and an index computed from the equation:

$$\text{stimulation index} = (\text{stimulated } S + G_2)/(\text{unstimulated } S + G_2)$$

Clinical investigation

A total of 315 individuals was investigated and of these, 25 were controls. T lymphocyte cell cycle profiles, haematological, T and B profiles, and CMI tests were carried out on all candidates. Individuals with immune deregulation were treated with autogenous lymphocytic factor (ALF). These individuals were chemically sensitive and chronically ill. The illnesses include dermatitis, vasculitis, asthma, organic brain syndrome, and Gulf war syndrome. In all cases, these illnesses were characterized with immune system suppression, dysfunction or deregulation.

Major symptoms presented by these patients include one or more of the following symptoms: lacrimation, pruritis, swelling and puffiness (ocular); fullness, noise, and dizziness (otic); congestion, sneezing, rhinorrhoea and blowing (nasal); lump, clearing and postnasal drip (throat); hypersensitivity reactions (immune); arthritis and arthralgia, fatigue and muscle pain (musculoskeletal); pressure and cough (chest); weight loss and fatigue (constitutional); miliary, ethmoidal and frontal (headache); insomnia, shortness of breath and depression (neurological).

All 290 chemically sensitive individuals presented a history of being affected by environmental incitants found in categories such as food, biological inhalants, and chemicals. They presented histories of varied backgrounds, but common among them was that all showed irregular cell cycles and abnormal T lymphocyte profiles in both numbers and functions in T and B lymphocytes and subsets. DNA histograms showed over or under accumulation of various subtypes of lymphocytes in one or more phases of the cell cycle of each individual.

Cell-mediated immunity

Delayed cutaneous hypersensitivity, or cell-mediated immunity (CMI) responses were evaluated in 190 patients; the results were recorded before and after treatment with ALF. The multitest CMI test kit (Mérieux Institute, Miami, Florida) was used. Each kit contained seven antigens: tetanus, diphtheria, candida, proteus, streptococcus, trichophyton, and tuberculin. The tests were evaluated and read at 48 h. Evaluation involves scoring the size, and number of increase of the wheals. The diameter of each induration was measured in millimetres and averaged. A reaction was considered positive if the average diameter was 2 mm or more.

Comparative evaluation of T and B lymphocytes

T and B lymphocytes and subsets were evaluated flow cytometrically before and after treatment with ALF.

Table 1 Representative profile of symptoms and signs after autogenous lymphocytic factor treatment

Symptoms	Improvement: Number	%	No change: Number	%	Deterioration: Number	%	Total patients
Hypersensitive reaction	63	63	33	33	1	1	100
Recurrent infection	38	57	29	43	0	0	67
Fatigue	60	68	27	31	1	1	88
Lack of concentration	43	54	36	46	0	0	79
Arthritis	19	44	23	54	1	2	43
Gastrointestinal upset	29	40	43	60	0	0	72
Headache	28	44	33	53	2	3	64
Depression	42	58	30	42	0	0	72

Establishment of therapeutic dosage of ALF

In vitro and *in vivo* procedures were used to establish dosage. Blastogenesis was the *in vitro* test employed for establishing dosage in general. *In vitro* grown peripheral lymphocytes were challenged comparatively with the known mitogens phytohaemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) of varied concentrations. A dose responsive curve was established to evaluate the efficacy of ALF with that of the mitogens; 0.1 ml of 1:10 dilution of ALF was found to be optimal when administered twice weekly. Intradermal test was carried out on all recipients depending on their response and sensitivity. If unusual sensitivities were observed, the ALF was further diluted. This procedure was successively repeated until no adverse reaction was observed.

Results

Significant changes were observed within 1 to 6 weeks in patients treated with ALF. Changes were observed in immune regulation and overall clinical manifestations. With regard to clinical manifestations, there were noteworthy improvements (Table 1), although minimal symptoms continued after approximately 3 weeks of continued therapy.

In a normal cell cycle, the highest percentage of lymphocytes should be in the G_0 to G_1 phase (Figure 1). This percentage will change dramatically when these lymphocytes are stimulated by various incitants (Figures 3a and 3b). Consequently, different percentages will appear in the S and G_2M phases, producing a deregulated profile (Figures 1 to 4). Treatment with ALF regulates the T lymphocyte cell cycle profile.

Immunologically, there were significant regulations of T lymphocyte cell cycles, especially from one phase of the cycle to another. Patients became less sensitive and more tolerant to specific incitants. As treatment continued, in general in about 6 weeks, a more drastic shift toward that of a normal profile was observed. Figures 1 to 4 summarize the regulatory changes of some of the cell cycles studied.

Changes were observed in the profiles of the T and B cells where T and B lymphocytes and their subsets were evaluated before and after treatment. There was a significant ($p < 0.01$) change in the total lymphocyte count and subsets, T_4, T_8 in 92 patients investigated. It should be noted that ALF seems to act as an immune modifier since the total lymphocytes, T_4 and T_8 were significantly elevated or reduced in order to maintain normalization (Tables 2 and 3).

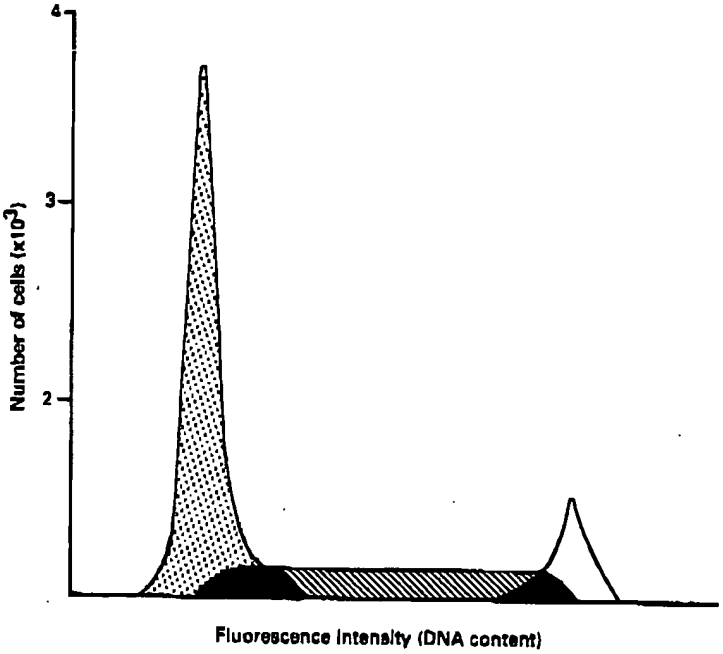

Figure 1 Diagrammatic representation of a normal mammalian cell cycle, showing the relative number of lymphocytes in each phase of the cycle, as displayed by the intensity of fluorescence of the fluorochrome-bound DNA in each lymphocyte. Normally, the percentage of lymphocytes in each phase of the cell cycle is 90–95, 5–10 and 0–5 in the G_0G_1 phase, S phase and G_2M phase, respectively. These quiescent lymphocytes display, normally, a low level of replication, and progression through the cell cycle. In response to stimuli, these cells undergo rapid division and cycle rapidly. The shadings show that there is overlapping of phases and not specific lines of demarcation: stippled, G_0G_1 phase (90–95% noncycling lymphocytes); hatched, S phase (5–10% cycling lymphocytes); unshaded (open), G_2M phase (0–5% cycling lymphocytes); and solid areas indicate overlapping of phases.

Table 2 Representative changes in T and B cell profiles after 92 chemically sensitive patients were treated with autogenous lymphocytic factor

Treatment	Total lymphocytes	T_{11}	T_4	T_8	T_4/T_8	B_4
Before	2,112 ± 632	1,624 ± 457	930 ± 45	439 ± 58	2.3 ± 0.8	188 ± 102
After	2,232 ± 678*	1,634 ± 544	1,027 ± 297**	478 ± 189**	1.3 ± 7.4	171 ± 120

* $p < 0.05$; ** $p < 0.01$; $n = 92$.

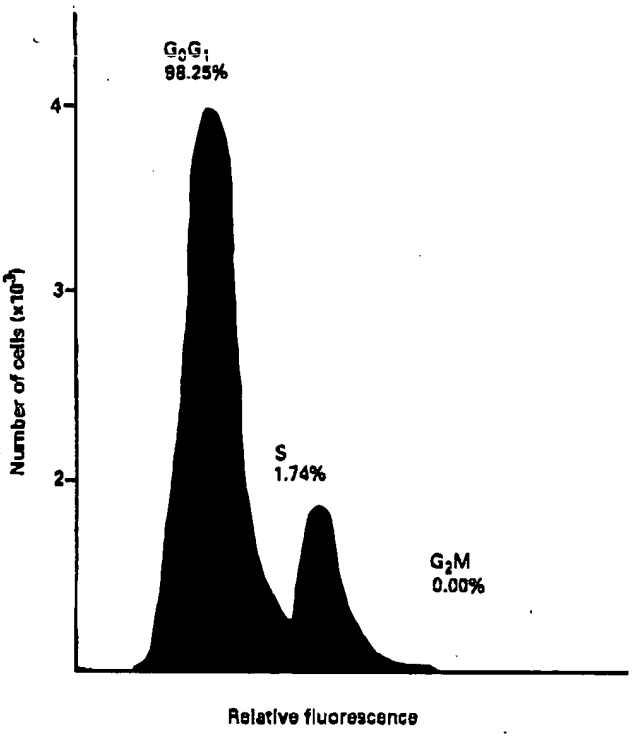

Figure 2 A representative DNA histogram of peripheral T lymphocytes from normal volunteers. Note that the highest percentage of lymphocytes is in the G_0G_1 phase, as expected, but the percentage varies in different individuals in keeping with the prevalence of environmental pollutants. Thus, the ideal normal profile is seldom achieved. The abscissa shows the relative fluorescence of the fluorochrome-bound DNA in each cell.

Table 3 Profile of T lymphocyte subsets modulation after treatment with autogenous lymphocytic factor

After treatment	Total lymphocytes: Number	%	T_{11} Number	%	T_4 Number	%	T_8 Number	%
Increase	52	57	41	46	53	53	55	60
Decrease	40	43	51	54	39	42	37	40
Probability	<0.05		>0.05		<0.05		<0.01	

Table 4a Typical cell-mediated response in (number and size) chemically sensitive individuals treated with autogenous lymphocytic factor

Patients	Before ALF	After ALF	P
190	5.46 ± 5.81	9.28 ± 7.25	<0.001

Results are given as mean ± SD.

Patients showed significant improvement ($p < 0.001$) in their CMI scores (Table 4). The regulatory effect of the immune system can be objectively assessed periodically after the initial treatment with ALF. The resourceful parameters are profiles of T lymphocyte cell cycle, T and B lymphocytes, and their subsets, cell-mediated immunity, signs and symptoms.

Side effects of ALF

The side effects were minimal and occurred only in six adults (five females and one male) where the average age was 52 years. These patients were intolerant of ALF, their symptoms included pain and irritation in the throat, burning in the eyes, pain and irritation in the chest, heat palpitations, influenza-like symptoms, headache, fatigue, and chills.

Table 4b Cell-mediated immunity positive score (number and size) after treatment with autogenous lymphocytic factor

Score	Patients	%	P
Increase	68	74	<0.001
No change	9	12	
Decrease	11	14	

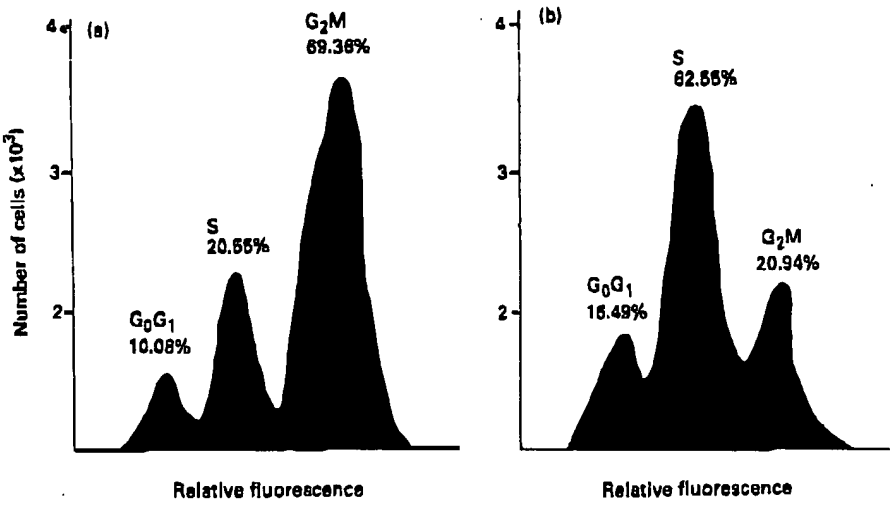

Figure 3a A representative irregular T lymphocyte cell cycle profile showing the effect(s) of stimulating the environmentally ill patients influence, primarily, the lymphocytes in the S phase.

Figure 3b A representative irregular T lymphocyte cell cycle profile showing the effect(s) of the stimulating incitants on the lymphocytes in the G_2M phase. It seems that each incitant, or a mixture of incitants affects lymphocytes in a particular phase(s) of the cell cycle, resulting in a variety of irregular cell cycle profiles and presumably dictates varied patterns of clinical manifestations. The abscissae in Figures 3a and 3b show the relative fluorescence of the fluorochrome-bound DNA in each cell.

Discussion

The flow cytometric profile of the T lymphocyte cell cycle as demonstrated by DNA histogram presents a reflection of the status of T lymphocytes in an individual. Of great importance is that its application is not limited to a certain category of individuals, but to normal subjects as well as individuals who are compromised by varied incitants. The essence of its clinico-biological importance, as detected in the present investigation, is that it is a reflection of T lymphocyte cell function, and may facilitate an in-depth approach to the treatment of some immunodeficient illnesses.

The progression of T lymphocytes from one phase of the cell cycle to another is time-dependent, ~8 to 12 h in the G_0-G_1 phase, 6 to 8 h in the S phase, and 0.5 to 1 h in the G_2M phase. The 290 individuals who were investigated in this study were affected principally by environmental incitants in categories such as foods, biological inhalants, and chemicals. They presented histories of varied backgrounds and different cell cycle profiles. The DNA histogram

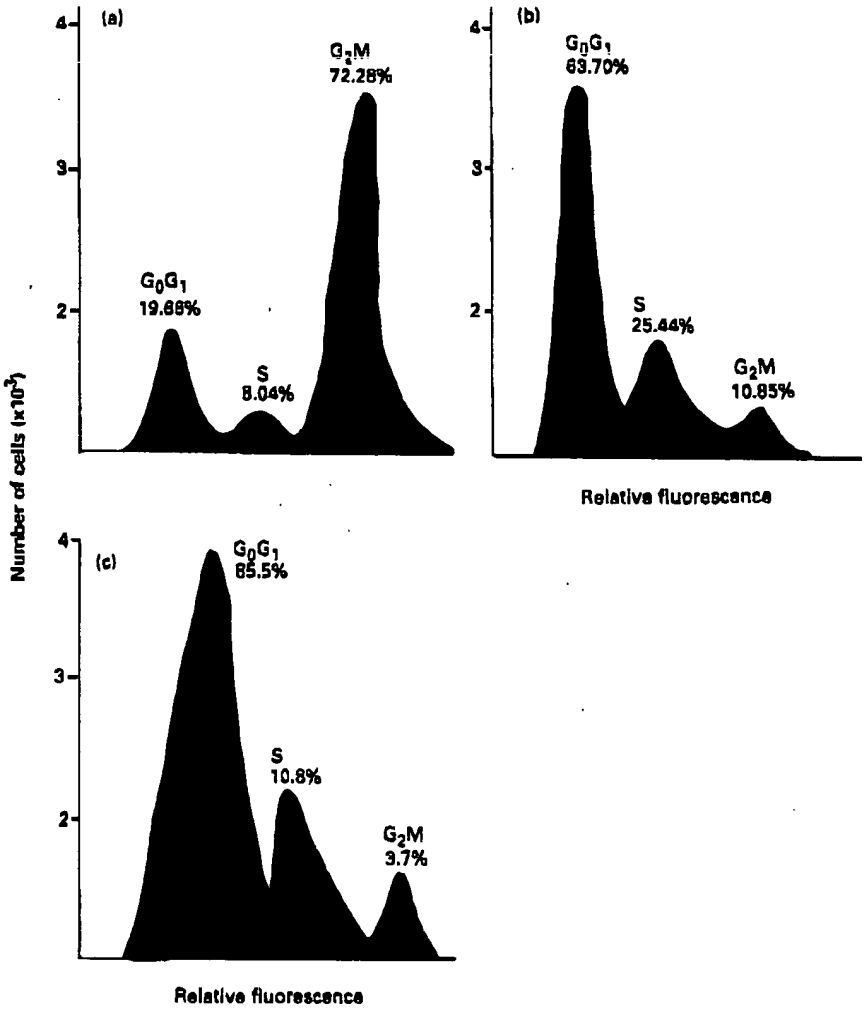

Figure 4a Initial T lymphocyte cell cycle profile of a patient on examination at the clinic. Note that the irregularity of the profile is emphasized in the G_2M phase.

Figure 4b Cell cycle of the patient in Figure 4a after the subject was treated with autogenous lymphocytic factor (ALF) for 3 weeks. Note that the percentages of lymphocytes in each phase of the cell cycle are still irregular, but there has been a significant improvement. The highest percentage of lymphocytes now appears in the G_0G_1 phase.

Figure 4c T lymphocyte cell cycle of patient (Figures 4a and b) subsequent to 3 months treatment with ALF. Note that the cell cycle has attained a relatively normal profile. ALF seems to act as a biological response modifier. The abscissae in Figures 4a, b and c show the relative fluorescence of the fluorochrome-bound DNA in each cell.

cycles showed over or under accumulation of lymphocytes in one or more phases of the cell cycle of each individual (Figures 3a and 3b). As shown in Figure 2, even the normal controls do not present an ideal DNA histogram profile of T lymphocytes. This is due to the fact that, in general, the ideal environment is seldom ever achieved, thus there is always some degree of immunological compromise to most individuals. It is reasonable then to assume that the incitant(s) which lead to immuno-incompetence of these individuals are capable of inactivating the particular enzyme(s), cyclin-dependent kinases, or depleting specific cyclins whose combination with their specific kinases are instrumental in catalysing the progression of T lymphocytes from one phase to another of the cell cycle. The T lymphocytes of the individual affected are locked in a particular phase, either resting, synthesizing, or multiplying too much in the G_0-G_1, S, G_2M phase respectively. Thus the subject manifests symptoms peculiar to the phases(s) affected. This hypothesis offers an opportunity to associate clinical manifestations with T lymphocyte cell cycle irregularity.

The treatment of lasting importance would be reasonably thought of as a biological response modifier, which would stimulate CDKs, regulate the cell cycle and the enzymes of purine and pyrimidine nucleotide synthesis. It is now generally accepted that these enzymes are elevated during the S phase (Cory, 1993). It seems logical that ALF stimulates these regulatory functions. The autogenous lymphocytic factor is a dialysable mixture of the many effector substances which may be released from *in vitro* grown stimulated lymphocytes with the ability to invoke immunological influences *in vivo* or *in vitro*, (Griffiths and Rea, 1995). The expected biological activity(s) of ALF would be to act as a biological response modifier, with mechanisms of action as suppressor and regulator of the immune system, especially in the regulation of the T lymphocyte cell cycle.

Kamp *et al.* (1994) showed that progression through the cell cycle requires the joint influence of positive regulator subunits, cyclins, and cyclin dependent kinases. These subunits regulate by phosphorylation a number of key substrates which subsequently activate a transition from G_1 to S, and G_2 to M phases of the cell cycle. Phosphate groups are transferred from ATP to a special amino acid in the target protein by protein kinases, while phosphatases remove the phosphate groups from the target proteins. The addition and removal of phosphate groups significantly affect the biochemical behaviour of the target proteins. Many protein kinases and phosphatases have a specific affinity for their target proteins, and act as determinants for controlling the activity(s) of their target

proteins. Indeed, ALF may possess these protein kinases which, acting as molecular switches, regulate an irregular T lymphocyte cell cycle to that of an ordered and orderly progression.

As demonstrated (Figure 4c), ALF regulates deregulated cell cycles, improves immunological profiles by increasing or decreasing the number of circulating lymphocytes and their subpopulations (Tables 2 and 3); and restores immune responses as demonstrated by enhanced cutaneous hypersensitivity (CMI) (Table 4). ALF also invoked immediate intradermal test response within 45 min of administration subsequent to the administration of antigen challenges which were previously negative or delayed for at least 15 days. Responses were observed by the number and size of wheals to the antigens which were inoculated. It is noteworthy that some chemically sensitive patients responded favourably, or completely recovered after environmental exposure to air, food, water, nutritional support and exercise. Some patients responded but did not fully recover. The patients who recovered reacted profoundly to exposure of very minute environmental insults of ambient chemicals, biological inhalants, and foods, or inhalants. However, when these patients were treated with ALF, their hypersensitivity was markedly reduced or disappeared; there was also significant improvement in recurrent infection, fatigue, headaches, depression, concentration, and gastrointestinal upsets. Eight patients immediately improved on initial injection with ALF.

T-suppressor, T-helper cells, and total lymphocytes were increased as reflected in an increase in a low population, and a decrease in a too high population. This suggests that ALF is a modulator rather than a stimulator. The mechanism(s) of action of ALF have not yet been ascertained. Indeed, the purification and determination of ALF from sorted T lymphocytes with or without helper phenotypes will be necessary to facilitate understanding the mechanism of action. However, it has been documented that the human cells contain a regulatory protein, CKS protein, which is a genetic suppressor of temperature sensitive CDK mutations. There are two isoforms of this CKS protein, namely CKSH1 and CKSH2. It is believed that CKSH2 protein binds to the catalytic subunit of the CDKs, and is essential for their biological function (Parge *et al.*, 1993). To this end, further investigations are being carried out.

The concept of a functional cell cycle is timely. Indeed, the profile of a cell cycle offers an insight into the immune status of a patient. However, the cell cycle profile does not indicate whether or not the lymphocytes are immunologically responsive or capable of responding to therapy. The influence of varied environmental incitants and/or

other systemic illnesses may render these lymphocytes incapable of normal progression through the cell cycle, thus resulting in a non-functional cell cycle. *In vitro* lymphocyte activation represents a standard procedure for evaluating cell mediated responses to a variety of stimuli including antibodies, polyclonal mitogens, specific antigens and cytokines. Interleukin-1 alpha (Il_1 alpha) and ALF were used as activators in the present investigation. Interleukin-1 alpha was used due to its extremely broad spectrum of bioactivity. ALF was used to observe its capability as a biological response modifier. The stimulatory process presented in this investigation offers a method of computing lymphocyte stimulatory response, and consequently an opportunity to establish an individual regime of treatment of illnesses associated with immunodeficiency. This investigation offers: (1) a vivid picture of the status of the immune profile of a patient as demonstrated by the DNA histogram of the T lymphocyte cell cycle; (2) an opportunity to regulate an irregular T lymphocyte cell cycle by treatment with autogenous lymphocytic factor; and (3) a clinical tool for the regulation of abnormal cell cycles and/or haematological and immunological profiles in humans with immunological deregulation. It should be noted that there is a pending United States patent, number 08/380,063 for the application of the cell cycle to clinical medicine, and also the regulation of the cycle by autogenous lymphocytic factor.

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Youdim et al.
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Griffiths et al.
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TREATMENT OF ENVIRONMENTALLY SENSITIVE PATIENTS WITH TRANSFER FACTOR PART II: CLINICAL STUDIES AND IMMUNOLOGICAL CORRELATES

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William J. Rea, M.D.[2]

ABSTRACT

Fifty patients ranging in age from 7 to 75 with hypersensitivity reactions to foods, inhalants and chemicals having symptoms of cephalgia, recurrent infections, fatigue, lack of concentration, arthritis, gastrointestinal problems and depression were treated with a course of transfer factor one unit two times per week for a period of 6-12 months. Seventy-eight percent of the patients had some relief as indicated by decrease in both the severity and frequency of one or more of their symptoms. These clinical improvements correlated well with increased cutaneous delayed type hypersensitivity (cellular immune response) (73.5%), as well as increase in the total number of lymphocytes (66.7%), total T cells (66.7%) and T helper cells (70.4%). These data indicate that antigen specific and possibly non-specific components of TF may modify the immunologic response of TF recipients and improve their clinical status. *Transfer factor, cellular immune reponse, T & B Lymphocytes.*

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INTRODUCTION

The clinical use of transfer factor (TF) in the therapy of a wide variety of disorders is well documented (1,2). These include congenital immunodeficiencies, acquired immunodeficiencies, malignancies, autoimmune and neurological, as well as infectious diseases such as protozoal, mycobacterial, fungal and viral diseases. In most cases the effect of TF on such infections is prophylactic and may result in either the initiation or restoration of cellular immunity. Lawrence reported that sixty-six percent (24/36) of patients with congenital immunodeficiency given TF converted to positive cutaneous delayed type hypersensitivity (DTH), and/or produced migration inhibitory factor (MIF), and/or responded to the corresponding antigens by lymphocyte transformation. In addition, 44% (16/36) of his patients improved clinically (1). In these studies, the transfer of an antigen-specific moiety, as well as nonspecific components in the TF preparation, was demonstrated. In fact, some studies (3,4) have shown that at least 130 different moieties are present in the extract prepared by the method of Lawrence. Fudenberg, et. al. (5) described some of these as a nonspecific adjuvants of cell mediated immunity while other components such as hypoxanthine, may be inhibitory.

We treated 50 environmentally sensitive patients, some with immune dysregulation, with a preparation of TF of molecular weight up to 30,000 Dalton units. Since the antigen-specific component of TF is reported to have a molecular weight between 3,500 and 12,000 Dalton units (6), it can be safely assumed that the above preparation also contained nonspecific components.

MATERIAL AND METHODS

Preparation of transfer factor (TF), lymphocyte phenotyping, and performance of cell mediated immunity (CMI) tests were as described in the accompanying paper (7).

Patient Selection: Patients with food allergies (8,9),

chemical, and environmental sensitivities (10) were advised to adopt environmentally safe habits such as living in a natural gas-free environment and to use less chemically contaminated food and water. The majority of patients also took antigen immunotherapy for foods, inhalants, or chemicals (8,11,12) concurrently with TF.

Patient Questionnaire: Each patient was given a "Symptoms Score Sheet" to be filled in prior to and following 6-12 months of TF therapy. The patients were asked to respond as to the frequency and severity of symptoms in the following categories: hypersensitivity reactions to incitants, cephalgia, recurrent infections, fatigue, gastrointestinal problems, depression, and lack of concentration. Based on the patient's response on a scale of 1 to 5, each respondent was categorized as demonstrating moderate decrease, substantial decrease, (i.e. improvement), or no change in his/her symptoms. The graded scores of frequency and severity of symptoms were evaluated before and after therapy.

TF Dose: Each patient received two weekly doses of TF each, injected subcutaneously or intramuscularly for 6-12 months. Each dose of 1 ml contained 10^8 lymphocyte equivalents.

RESULTS

Fifty patients were entered into a program of TF therapy for 6 to 12 months. Table 1 shows the distribution of age and sex among these patients. There were a total of 8 males and 42 females ranging in age from 7 to 75 years. Each patient was asked to submit a symptoms score before and after TF therapy. Tables 2 and 3 summarize data generated from these questionnaires. Depending on each symptom category, patients showed either a moderate decrease, substantial decrease, or no change in the severity or frequency of their symptoms. A substantial number of patients admitted to some relief as indicated by decrease in both severity and frequency of their symptoms.

We next examined the correlation of improved clinical status to the increase in the number of WBC, lymphocytes, T cells, T cell subpopulations, B cells and CMI response in TF recipients. These data are given in Tables 4 and 5. 39 of 50 patients (78%) responded to TF therapy with some improvement in one or more of their symptoms (Tables 2, 3 and 5). The closest immunologic correlate of improvement in clinical status was the patient's CMI response (73.5%), followed by increase in the number of T helper cells (70.4%). Both improvement in CMI and T helper cell numbers show excellent correlation to the decrease in the frequency of recurrent infection of 70.6% (Table 3). In every case, an increase in the number of WBC, total number of lymphocytes, T11, T4, T8, and B lymphocytes were in good agreement with improvement in clinical status.

Not all patients, however, responded well to TF therapy. In fact, 22% of the patients demonstrated no improvement in their clinical status and a minor population showed no change or a decrease in their immune parameters (Table 4).

The cumulative data indicates that a substantial portion of the patients (73.5%) exhibited an increase in their CMI, in the number of their B and T lymphocytes (62.1%) or in both of these parameters (95.5%) (Table 4).

TABLE 1
The Distribution of Age and Sex
Among 50 Patients Treated with TF

AGE/SEX	MALE	FEMALE	TOTAL
01-10	1	00	01
11-20	1	01	02
21-30	2	02	04
31-40	1	14	15
41-50	3	10	13
51-60	0	11	11
61-70	0	03	03
71-80	0	01	01
TOTAL	8	42	50

DISCUSSION

The results presented in this paper (Tables 2 and 3) indicate improvements in eight clinical parameters reported by the patients enrolled in a program of TF therapy for 6 - 12 months.

A large number of the patients concurrently utilized antigen immunotherapy (8,11,12) and had adopted environmentally less-toxic or less-allergenic living habits. It can also be seen that these same patients were, for the most part, middle-aged females who were long-term referrals at the Environmental Health Center - Dallas (EHC-Dallas).

Improvement of 60% to 70% in various symptom categories by these patients closely parallelled enhanced immunologic parameters (Tables 4 and 5) These results clearly resemble previously published clinical improvement of 44% and in vitro and in vivo cellular immune responses by 66% of congenitally immunodeficient patients treated with TF (1). An interesting question posed at this juncture is the relative contribution of the specific and nonspecific components of the TF preparation. The chemical complexity of the TF preparation (with over one hundred different molecules) makes it difficult to assign specific functional activity to each molecule. Resolution of this

TABLE 2
Improvement in Clinical Severity of Symptoms Indicated by
Patients Receiving Transfer Factor Therapy

SEVERITY

	Moderate Decrease		Substantial Decrease		No Change		Total # Patients Improved	Total # Patients Tested	Patients Improved %
	#	%	#	%	#	%			
Hypersensitivity Reactions	27	54	6	12	17	34	33	50	66
Cephalgia	13	31.7	9	21.95	19	46.3	22	41	53.7
Recurrent Infection	16	36.4	10	22.7	18	40.9	26	44	59.1
Fatigue	19	38.8	15	30.6	15	30.6	34	49	69.4
Lack of Concentration	16	37.2	12	27.9	15	34.9	28	43	65.1
Arthritis	9	33.3	3	11.1	15	55.6	12	27	44.4
G.I. Problems	12	41.4	3	10.3	14	48.3	15	29	51.7
Depression	12	37.5	7	21.9	13	40.6	19	32	59.4

TABLE 3
Improvement in Frequency of Symptoms Indicated by
Patients Receiving Transfer Factor Therapy

FREQUENCY

SYMPTOMS	Moderate Decrease		Substantial Decrease		No Change		Total # Patients Improved	Total # Patients Tested	Patients Improved %
	#	%	#	%	#	%			
Hypersensitivity Reactions	16	35.6	10	22.2	19	42.2	26	45	57.8
Cephalgia	07	20.6	12	35.3	15	44.1	19	34	55.9
Recurrent Infection	14	41.2	10	29.4	10	29.4	24	34	70.6
Fatigue	14	31.8	13	29.5	17	38.6	27	44	61.3
Lack of Concentration	16	41	11	28.2	12	30.8	27	39	69.2
Arthritis	08	30.8	6	10.7	17	60.7	14	26	53.8
G.I. Problems	08	28.6	03	10.7	17	60.7	11	28	39.3
Depression	10	32.3	08	25.8	13	41.9	18	31	58.1

TABLE 4
Changes in Immune Parameters After Transfer Factor Therapy

Immune Parameters	# Patients Tested	Below Normal Range Change to Normal Ranges		Partial Increase Towards Normal Range		Total Increase in Range		No Change		Decrease	
		#	%	#	%	#	%	#	%	#	%
CMI*	34	12	35.3	13	38.2	25	73.5	05	14.7	04	11.8
T&B**	29	09	31.03	09	31.03	18	62.1	09	31.03	02	06.9
CMI & T&B	22	09	40.9	12	54.5	21	95.5	01	04.5	0	0

CMI in Normal Population

	Mean # Reactions	Mean Reaction Size
315 Male	4.5	18.3
299 Female	3.5	12.2

*Data provided by Institute Merieux, Lyon, France
**Normal ranges established on 60 males and females by EHC-Dallas

difficulty will have to await the isolation and purification of each indigenous molecule. Such components as thymosin A-1 like materials (13), Interferon (14), prostaglandins (15), serotonin, ascorbate, chemoattractants and immobilizing factors (1,5) must exert considerable immunological, biochemical, hormonal and other physiological stimuli on the TF recipient. It is well known, for example, that interferon (16) exhibits at least some 15 enzymatic and biochemical properties and 27 immunoregulatory functions. The anti-viral (17,18) and antiproliferative (19) effects of interferon are well known and could be responsible for decreased recurrent infections. Thymosin A-1 like materials (13) enhance Migration Inhibitory Factor (MIF), Alpha and Gamma interferon, Interleukin-2, and Lymphotoxin production, and stimulate viral, fungal and tumor immunity and increases IL-2 receptor expression as well as amplify T-cell immunity in humans (20,21).

All of these effects modulate biological and immunoregulatory functions, and may be responsible for the changes observed in our patients. Prostaglandins (PG), another component of TF (15), are a family of biologically active compounds (22) and products of arachidonic acid metabolism via a cyclo-oxygenase dependent pathway. These compounds are vital components of the balance achieved by normal immunoregulatory mechanisms and implicated in inflammatory reactions, via PG-producing monocytes. PGE2 modulates T-cell activation, T-cell response to antigen, lymphoxine production, B-cell and natural killer cell activity (23). Serotonin and histamine are bioactive amines (24,25) and components of the immediate or type I allergic reaction.

TABLE 5
Correlation of Increase in the Number of Lymphocytes and CMI Response Versus Clinical Status After Transfer Factor Therapy

	# Patients Tested	# Patients Improved	%
WBC	27	18	66.7
LYMPHOCYTES	27	18	66.7
T11	27	18	66.7
T4	27	19	70.4
T8	27	15	55.6
T4/T8	27	07	25.9
B LYMPHOCYTES	24	14	58.3
CMI	34	25	73.53
CLINICAL STATUS*	50	39	78.0

*Based on Patient response to:

Hypersensitivity Reactions	Arthritis	Cephalgia
Lack of Concentration	GI Problems	Depression
Recurrent infection	Infection	Fatigue

It is, therefore, possible that both specific (1) and nonspecific modulators (26,27) or "nonimmunologic" components of TF contribute to the improvements observed in the recipients of TF. Although an interesting question, the contribution of each individual moiety will have to await further research. Suffice to say that pooled normal TF, comprised of random subsets of specificities, is a valuable therapeutic adjunct for treatment of environmentally ill patients.

Finally, we should mention that TF was not universally tolerated. A small number of patients were taken off therapy for nonspecific complaints, as well as muscle and joint aches, "cold or flu-like" symptoms, slight fever and local edema or pain at the site of injection. Most of these effects may be attributed to interferon (16) and were transitory in nature.

Alternatively, immunogenetic factors, extreme sensitivity of the patients, quality of the TF preparation or psychologic factors may have played a role in patient intolerance. For the majority of the patients, however, TF was without complications and quite safe. In a double-blind study (Fudenberg and Segre, private communications), showed that long-term administration of TF to mice from time corresponding to middle age until time of death, had no adverse effect upon life span, in fact, mean, median, and maximum life span was the same for the mice that received TF as for those that received saline. Ottgen, et.al.(1, 28) injected pooled TF into women with breast cancer. Doses as large as 257 ml (equivalent to 217 billion cells) were given repeatedly (125 injections) over a period of 310 days. This immunotherapeutic approach testifies to the margin of safety that is afforded by transfer factor.

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AUTHOR: **REA W J**; WILLIAMS M L; SPRAGUE D E
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The role of the T lymphocytic cell cycle and an autogenous lymphocytic factor in clinical medicine.

AUTHOR: Griffiths Bertie B(a); **Rea William J**; Griffiths Bradley; Pan Y
AUTHOR ADDRESS: (a)EHC-D Analytical Lab., Environ. Health Cent.-Dallas, 8345 Walnut Hill Lane, Suite 240, Dallas, T, USA

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Abstract: The term functional somatic syndrome has been applied to several related syndromes characterized more by symptoms, suffering, and disability than by consistently demonstrable tissue abnormality. These syndromes include multiple **chemical sensitivity**, the sick building syndrome, repetition stress injury, the side effects of silicone breast implants, the Gulf War syndrome, chronic whiplash, the chronic fatigue syndrome, the irritable bowel syndrome, and fibromyalgia. Patients with functional somatic syndromes have explicit and highly elaborated self-diagnoses, and their symptoms are often refractory to reassurance, explanation, and standard treatment of symptoms. They share similar phenomenologies, high rates of co-occurrence, similar epidemiologic characteristics, and higher-than-expected prevalences of psychiatric comorbidity. Although discrete pathophysiologic causes may ultimately be found in some patients with functional somatic syndromes, the suffering of these patients is exacerbated by a self-perpetuating, self-validating cycle in which common, endemic, somatic symptoms are incorrectly attributed to serious abnormality, reinforcing the patient's belief that he or she has a serious disease. Four psychosocial factors propel this cycle of symptom amplification: the belief that one has a serious disease; the expectation that one's condition is likely to worsen; the ''sick role,'' including the effects of litigation and compensation; and the alarming portrayal of the condition as catastrophic and disabling. The climate surrounding functional somatic syndromes includes sensationalized media coverage, profound suspicion of medical expertise and physicians, the mobilization of parties with a vested self-interest in the status of functional somatic syndromes, litigation, and a clinical approach that overemphasizes the biomedical and ignores psychosocial factors. All of these influences exacerbate and perpetuate the somatic distress of patients with functional somatic syndromes, heighten their fears and pessimistic expectations, prolong their disability, and reinforce their sick role. A six-step strategy for helping patients with functional somatic syndromes is presented here.
Identifiers--KeyWord Plus(R): CHRONIC-FATIGUE-SYNDROME; IRRITABLE-BOWEL-SYNDROME; RANDOMIZED CONTROLLED TRIAL; TERM FOLLOW-UP; MULTIPLE CHEMICAL SENSITIVITIES; POSTTRAUMATIC-STRESS-DISORDER; COGNITIVE-BEHAVIORAL THERAPY; MASS PSYCHOGENIC ILLNESS; CARE-SEEKING BEHAVIOR; UPPER LIMB PAIN
Cited References:
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Annual Meeting of American Academy of Otolaryngic Allergy (ARTICLE)

Archives of Otolaryngology
Apr, 1996; Medical News: tzo446
LINE COUNT: 00104
0003-9977

This meeting was held at the Ernest N. Morial Convention Center, New Orleans, La, on September 14 and 16, 1995.

John H. Boyles, Jr, MD, Dayton, Ohio, found in a review of the literature, 65 articles reporting allergy-related inner ear disease. He reported long-term results in 30 personal cases of patients with the classic symptoms of Meniere's disease. Of the patients, 35% had ahistory of allergies and 53% a family history of Meniere's disease, 47% were sensitive to foods, and 32% were sensitive to chemicals. Theworkup must include a complete evaluation of inhalants, foods, chemicals, fungal disorders, autoimmune disorders, and nutrition. Eleven of the 30 patients exhibited autoimmune thyroid disorder.

David Morris, MD, La Crosse, Wis, reviewed several double-blind, placebo-controlled studies in southern Europe that demonstrated significant effectiveness of sublingual therapy with grass and house-mite antigens. Recent immunologic studies, which support sublingual treatment, show that pure antigens that reach the bloodstream without adjuvant are, in general, tolerogenic. It is believed that the direct effect on suppressor cells accounts for the positive results in immediate and delayed reactions.

William J. Rea, MD, Richardson, Tex, using flow cytometry, producedDNA histograms showing normal and abnormal cell cycles that indicate immune status. An immune modulator, called **autogenous lymphocyte factor** (ALF), was produced from autogenous cell cultures. The ALF wasinjected in 101 patients with treatment-failure chemical sensitivity,who had abnormal T cells and T-cell dysfunction. He reported 87% clinical improvement in these patients (P=.001). Only six could not tolerate ALF.

Eleven patients were treated with immunotherapy for allergic fungalsinusitis (AFS) by Richard L. Mabry, MD, Scott C. Manning, MD, and Cynthia S. Mabry, RN, Dallas, Tex. They reported on patients whose injections were advanced weekly for an average of 13 months. There were no adverse effects. After 4 to 8 weeks of immunotherapy, crusting was decreased.

William J. Rea, MD, Richardson, Tex, reported autonomic nervous system and immune system abnormalities in patients with silicone breast implants. The patients demonstrated symptoms as follows: musculoskeletal, 87%; neurological, 60%; gastrointestinal, 27%; cardiovascular, 24%; and otologic, 20%. Skin tests for silicone were positive in 68% of patients. Ninety percent of patients demonstrated serologic evidence of autoimmunity. Results of a pupillogram, which reads speed of contraction and dilatation of the pupil as evidence for autonomic dysfunction, was abnormal in 93%.

Andrew J. Lehr, MD, Richard L. Mabry, MD, and Cynthia S. Mabry, RN,Dallas, Tex, demonstrated that a nine-antigen `midiscreen' was more sensitive than a six-antigen 'miniscreen' and as sensitive as a 13-antigen screening radioallergosorbent test (RAST) to identify patients with significant allergy. The authors concluded that a nine-antigen midiscreen

STIC-ILL

240959

From: Schwadron, Ronald
Sent: Wednesday, February 10, 1999 11:02 AM
To: STIC-ILL
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Youdim et al.
Clin. Ecology
vol 7
p 55-61
1990

Youdim et al.
Clin. Ecology
vol 7
p 62-66
1990

his phone # 214-368-4132

Youdim et al.
Clin. Ecology
vol 7
p 67-72
1990

left message w/ secretary 2/18

Rea
Annual Meeting-American Academy of Environmental Medicine (I think this is a book of abstracts published by American academy of Environmental Medicine)
Sept 1995
p213-222

Env. Health Center of Dallas

Griffiths et al.
Cytobios
vol 93
p49-66
1998

s/w Ellie

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X/61

thanxs

* Please see attached note from the author.

3/1
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01474979 INSIDE CONFERENCE ITEM ID: CN014639864
The Potential Uses for Autogenous Lymphocyte Factors (ALF) in Environmental Medicine
Rea, William - owner of clinic
CONFERENCE: American Academy of Environmental Medicine: The cutting edge of environmental medicine-Annual meeting; 30th
ANNUAL MEETING- AMERICAN ACADEMY OF ENVIRONMENTAL MEDICINE, 1995; 30th P: 213-222
The Academy, 1995
LANGUAGE: English DOCUMENT TYPE: Conference Abstrcts, presentations, papers and programme
CONFERENCE SPONSOR: American Academy of Environmental Medicine
CONFERENCE LOCATION: Tucson, AZ
CONFERENCE DATE: Sep 1995 (19950) (19950)

520

2/3/2
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01474969 INSIDE CONFERENCE ITEM ID: CN014639768
Some Examples of Administrative Law in Action
Rea, W.
CONFERENCE: American Academy of Environmental Medicine: The cutting edge of environmental medicine-Annual meeting; 30th
ANNUAL MEETING- AMERICAN ACADEMY OF ENVIRONMENTAL MEDICINE, 1995; 30th P: 127-136
The Academy, 1995
LANGUAGE: English DOCUMENT TYPE: Conference Abstrcts, presentations, papers and programme
CONFERENCE SPONSOR: American Academy of Environmental Medicine
CONFERENCE LOCATION: Tucson, AZ
CONFERENCE DATE: Sep 1995 (19950) (19950)

ENVIRONMENTAL HEALTH CENTER-DALLAS
8345 Walnut Hill Lane, Suite 220, Dallas, Texas 75231

FAX

Date: 2/23/99

Number of pages including cover sheet:

To:
Linda

Phone:

Fax phone: 703/305-3585

CC:

From:
William J. Rea, M.D./Ellie

Phone: (214) 368-4132 373-5161

Fax phone: (214) 691-8432

REMARKS: ☐ Urgent ☐ For your review ☐ Reply ASAP ☐ Please comment

Instructions:
Linda: A paper was not published from the AAEM abstract. However, I am attaching a published paper regarding ALF.

The role of the T lymphocytic cell cycle and an autogenous lymphocytic factor in clinical medicine

Bertie B. Griffiths*, William J. Rea, Bradley Griffiths and Y. Pan

EHC-D Analytical Laboratory, Environmental Health Center – Dallas, 8345 Walnut Hill Lane, Suite 240, Dallas, Texas 75231, U.S.A. (*Reprint address)

Key words: cell cycle, flow cytometry, T lymphocytes, autogenous lymphocytic factor, chemical sensitivity

Abstract

In this study 315 individuals (25 controls, 290 chemically sensitive immunocompromised patients) were investigated. Each patient had been on a standard therapy of avoidance of pollutants, nutritional supplementation, and injections of antigens for foods, and biological inhalants, but did not attain their immunological competence. Peripheral lymphocytes were collected and DNA histograms were constructed. The flow cytometer was used to evaluate the cell cycle, haematological, and other immunological profiles. From the other portion of the blood specimen, lymphocytes were propagated *in vitro*, harvested, and a lysate, termed the autogenous lymphocytic factor (ALF), was prepared. When treated with ALF, 88% of these individuals showed a significant ($p < 0.001$) clinical improvement which correlated with laboratory findings, involving regulation of abnormal cell cycles, increase in total lymphocytes and subsets T_4, T_8, ($p < 0.05$) and cell mediated immunity (CMI) response ($p < 0.001$). The ALF presumably acts as a biological response modifier. The cell cycle and ALF provide clinical tools for diagnosis and regulation of immunological incompetence.

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Introduction

The cell cycle is the ordered and orderly events of biochemical and morphological sequences, leading from the formation of a daughter cell as a result of mitosis, to the completion of the processes required for its own division into two daughter cells (Lee and Dang, 1995). It is fundamental that following mitosis, two daughter cells are produced. These cells may initiate a new cycle (G_1 phase), some cells may become nonproliferative (G_0 phase) or progress to a restriction point where they are committed to the synthesis of cellular components, (the S phase) and finally complete the cycle (the G_2M phase). The cell cycle then comprises the sum of the growth phases of a specific cell cycle. This cycle is repeated by continuously dividing cells. Even within the same organism the specific cell cycle will vary with different classes of cells. The total time that is necessary to complete the S and G_2 phases is generally constant in different cell types. It seems reasonable then, to assume that most of the time variation takes place in the G_1 phase.

To establish homeostasis, it is imperative that the cell cycle be regulated. To this end, there are biological parameters which may be employed to ascertain the normality of a particular cell cycle. The DNA content in the nucleus of a cell (2 N or diploid amount) is constant in all normal organisms (Givan, 1992). There are only two exceptions where the DNA content is not constant; the amount of DNA will vary in cells which undergo meiosis in preparation for sexual reproduction, thus containing the 1 N or haploid amount of DNA. The other exception applies to those cells which undergo DNA synthesis in preparation for mitosis. These will contain between 2 N and 4 N amounts of DNA.

By employing the concept of DNA constancy in a particular organism, and the application of flow cytometric techniques, DNA flow histograms can be constructed depicting a normal cell cycle and the identification of abnormal cycles.

The regulation of the cell cycle in eukaryotes seems to take place at two main transition points, prior to DNA replication at a point in the G_1 phase, termed the restriction point, and prior to cytokinesis at the G_2M phase boundary (Nurse and Bissett, 1981). The progression of the cell cycle from one phase to the other is mediated by specialized proteins, cyclins, and the activation of enzymes called cyclin-dependent kinases (CDKs) and by a number of positive and negative feedback loops. To date seven CDKs have been identified and designated CDK 2 to 8 (Meyerson *et al.*, 1992; Lorinez and Reed, 1984; Nurse, 1994; Sherr, 1994; Helchman and Roberts, 1994; King *et al.*, 1994; Hunter and Pines, 1994). Each CDK acts at different stages of the cell cycle and is differentially regulated by different cyclins (Meyerson and Harlow, 1994). The critical roles played by cyclins in the regulation of eukaryotic cell cycle is amply documented.

These CDKs play very significant roles in the G_1S and G_2M transitions during the mammalian cell cycle. They regulate by phosphorylation, a number of key substrates which subsequently activate a transition from G_1 to S and from G_2 to M (Kamp *et al.*, 1994). The catalytic subunits alone of these CDKs are not active and require the influence of positive regulatory subunits to ensure biochemically active protein kinase holoenzymes (Desai *et al.*, 1992). The positive regulatory subunits employed to this end are cyclins. The activity of CDKs is, therefore, regulated by both cyclins and specific phosphorylation and dephosphorylation (Gold and Nurse, 1989; Draetca and Beach, 1988; Solomon *et al.*, 1990; Gutier *et al.*, 1991; Murray and Krischner, 1989).

Cyclins were identified originally as proteins in the murine invertebrate cells. The concentration of these cyclins accumulate and are destroyed periodically at defined points during the cell cycle (Evans *et al.*, 1983). At present, eight cyclins have been identified; these are designated A, B, C, D, E, F, G_1, and H based on their amino acid sequences (Pines and Hunter, 1989; Xiong *et al.*, 1991), and in some instances, on genetic complementation experiments in yeast (Nurse, 1990; Murray and Krischner, 1989; Lewin, 1990; Sherr, 1993).

The influences of the cyclins are expressed differently; *e.g.* cyclin A exhibits its influence through the S-M phase (Wang *et al.*, 1990; Fang and Newport, 1991; Walker and Maller, 1991; Lehner and O'Farrell, 1990; Pagano *et al.*, 1992; Zindy *et al.*, 1992) of most cells, while cyclin B (which is conserved from yeasts to humans) propels cells into mitosis (Nurse and Bissett, 1981; Evans *et al.*, 1983; Glotzer *et al.*, 1991). Both the A and B cyclins are degraded at the M phase by ubiquitin (Ub)-dependent proteolysis (Glotzer *et al.*, 1991). G_1 cyclins (CIN 1, CIN 2, CIN 3) are assumed to associate with a p34 CDC2 homologue, p34 CDC 28, driving yeast cells into S phase (Reed, 1992; Cross, 1988; Richardson *et al.*, 1989; Hadwiger *et al.*, 1989; Wittenberg *et al.*, 1990; Nasmyth, 1990).

In the Environmental Health Center - Dallas (EHC-D), clinical cases are investigated varying from surgical to environmental illnesses. However, cases involving chemical sensitivity entail the principal aspect of our practice. Over a period of 22 years our laboratory observations show that characteristically, a percentage of the chemically sensitive individuals portray a secondary deficit which is related to immune dysfunction, principally, depression of the suppressor T-cell (Rea *et al.*, 1986, 1987).

Initial profiles of lymphocyte cell cycle progression, T and B lymphocytes, cell mediated immunity and haematograms of patients were compared with those taken subsequent to treatment with autogenous lymphocytic factor (ALF). Acting presumably as a biological response modifier, ALF was observed to modulate deregulated immune profiles.

The focus of this investigation was firstly to observe the patterns of DNA histograms of the T lymphocyte cell cycles, especially in chemically sensitive individuals. The histograms provide a 'snapshot' of the individual's present cell cycle. By comparing an individual's cell cycle with the classic normal cycle, very crucial information will be obtained to enable a scientific regulation of a patient's cell cycle. Secondly the work was to prepare a lysate

from the *in vitro* propagated peripheral T lymphocytes of an individual with an irregular T lymphocytic cell cycle, and observe the regulatory effect of this lysate, when injected subcutaneously into such an individual. Thirdly, the intention was to establish a basis for the regulation of an individual's T lymphocytes which were observed to be irregular due to varied incitants, thus restoring normal T lymphocyte functions, and the enabling of a compromised individual to cope with multiple insults to his/her immune system. Fourthly, we examined the responsiveness of T lymphocytes to autogenous lymphocytic factor (ALF) as measured by the cell mediated immunity (CMI) test, and to establish on an individual basis an index of T lymphocyte ability to respond.

From our search of databases, and to the best of our knowledge there is no documentation pertaining to the regulation of the T lymphocytic cell cycle by lysates of an individual's T lymphocytes, and its application to clinical medicine.

Materials and methods

Isolation of lymphocytes

Venous blood was collected from 315 individuals in heparinized tubes. Of these 25 were healthy employees of EHC-D; these represented normal controls. The other individuals were chemically sensitive, chronically ill patients of the EHC-D and referrals from outside physicians. Erythrocytes were separated by a modified gradient technique using heparinized CPT vacutainers (Becton Dickinson Co.). This preparation was centrifuged for 30 min at 500 x *g*. The lymphocyte layer (interphase between isolymph and plasma) was removed to sterile polystyrene tubes. Lymphocytic layers were washed three times with normal saline (0.9% sodium chloride) by centrifugation in a refrigerated centrifuge at 500 x *g* for 20 min. Cells were resuspended in normal saline and counted.

Lymphocyte phenotyping

Monoclonal antibodies against pan T cells, T_{11} (CD2), T helper cells T_4 (CD4), T suppressor/cytotoxic cells T_8 (CD8), activated T cells TA1 (CD26) and pan B cells B_4 (CD19) were bought from Coulter Immunology (Hialeah, Florida). Peripheral lymphocytes were obtained by venipuncture, and stained by the use of Coulter Q prep Epics immunology station following manufacturer's instructions.

Preparation of cell cycles

Heparinized whole blood or lymphocytes were used and 100 µl aliquots of heparinized whole blood or 100 µl of washed isolated lymphocytes (suspended in normal saline) were placed in 13 x 75 mm tubes. To each tube was added 200 µl of lysing buffer (Coulter) and gently mixed for ~15 s, then 2.0 ml of DNA stain with RNAse was added and mixed for 20 s The mixture was stained with propidium iodide at a concentration of 50 mg per ml, and allowed a staining period of 15 min and then analysed by flow cytometry.

Flow micro-fluorometric analysis

Cells tagged fluorometrically for DNA content were analysed on the Coulter Epic C flow cytometer, where they were allowed to pass through a laser beam tuned to 488 nm. Fluorescence was measured electronically and recorded as a histogram. DNA distribution in the cell cycle was calculated on accumulated data by parametric analysis to produce a particular DNA histogram.

Preparation of autogenous lymphocytic factor (ALF)

Propagation of lymphocytes

To cell culture flasks, containing 10 ml RPMI 1640 medium and/or supplemented with 1 ml bovine calf serum was added 1 ml of washed T lymphocytes. The culture was incubated at 37°C and monitored daily for approximately 2 to 3 weeks.

Harvesting of cells

When the cell population approximated 5 to 9 x 10^6 per ml, culture suspensions were transferred to calibrated 50 ml conical tubes and centrifuged for 30 min at 500 x *g*. The supernate was discarded, cell pellet resuspended in 10 ml normal saline and washed three times by centrifugation for 30 min each time.

Preparation of ALF

Each cell pellet was resuspended in ~2.0 ml normal saline and sonicated at 20 W with a duty cycle of 50% for 60 s. The sonicated mixture was sterilized by filtration and stored at −20°C to be diluted for therapeutic use as indicated.

Establishment of a functional T lymphocyte cell cycle

The profile of an individual's T lymphocyte cell cycle may appear normal, yet the lymphocytes may not be functional. A process was established to evaluate the immunological responsiveness of the lymphocytes of the cell cycle.

Stimulation of peripheral lymphocytes

Nunclon™-plastic plates (24 wells) containing 2 ml of RPMI 1640 medium per well were inoculated with ~2 to 4 x 10^6 lymphocytes per ml, 1.25 ng interleukin 1 alpha (Il_1 alpha) per ml, or with 0.1 ml ALF per ml; negative controls had neither Il_1 nor ALF. The plates were incubated at 37°C for 96 h. Cells were harvested, washed and analysed by flow cytometrically as mentioned above. Comparison of the S and G_2 phases of both the unstimulated and the stimulated T lymphocytes was undertaken and an index computed from the equation:

$$\text{stimulation index} = (\text{stimulated } S + G_2)/(\text{unstimulated } S + G_2)$$

Clinical investigation

A total of 315 individuals was investigated and of these, 25 were controls. T lymphocyte cell cycle profiles, haematological, T and B profiles, and CMI tests were carried out on all candidates. Individuals with immune deregulation were treated with autogenous lymphocytic factor (ALF). These individuals were chemically sensitive and chronically ill. The illnesses include dermatitis, vasculitis, asthma, organic brain syndrome, and Gulf war syndrome. In all cases, these illnesses were characterized with immune system suppression, dysfunction or deregulation.

Major symptoms presented by these patients include one or more of the following symptoms: lacrimation, pruritis, swelling and puffiness (ocular); fullness, noise, and dizziness (otic); congestion, sneezing, rhinorrhoea and blowing (nasal); lump, clearing and postnasal drip (throat); hypersensitivity reactions (immune); arthritis and arthralgia, fatigue and muscle pain (musculoskeletal); pressure and cough (chest); weight loss and fatigue (constitutional); miliary, ethmoidal and frontal (headache); insomnia, shortness of breath and depression (neurological).

All 290 chemically sensitive individuals presented a history of being affected by environmental incitants found in categories such as food, biological inhalants, and chemicals. They presented histories of varied backgrounds, but common among them was that all showed irregular cell cycles and abnormal T lymphocyte profiles in both numbers and functions in T and B lymphocytes and subsets. DNA histograms showed over or under accumulation of various subtypes of lymphocytes in one or more phases of the cell cycle of each individual.

Cell-mediated immunity

Delayed cutaneous hypersensitivity, or cell-mediated immunity (CMI) responses were evaluated in 190 patients; the results were recorded before and after treatment with ALF. The multitest CMI test kit (Mérieux Institute, Miami, Florida) was used. Each kit contained seven antigens: tetanus, diphtheria, candida, proteus, streptococcus, trichophyton, and tuberculin. The tests were evaluated and read at 48 h. Evaluation involves scoring the size, and number of increase of the wheals. The diameter of each induration was measured in millimetres and averaged. A reaction was considered positive if the average diameter was 2 mm or more.

Comparative evaluation of T and B lymphocytes

T and B lymphocytes and subsets were evaluated flow cytometrically before and after treatment with ALF.

Table 1 Representative profile of symptoms and signs after autogenous lymphocytic factor treatment

Symptoms	Improvement: Number	%	No change: Number	%	Deterioration: Number	%	Total patients
Hypersensitive reaction	63	63	33	33	1	1	100
Recurrent infection	38	57	29	43	0	0	67
Fatigue	60	68	27	31	1	1	88
Lack of concentration	43	54	36	46	0	0	79
Arthritis	19	44	23	54	1	2	43
Gastrointestinal upset	29	40	43	60	0	0	72
Headache	28	44	33	53	2	3	64
Depression	42	58	30	42	0	0	72

Establishment of therapeutic dosage of ALF

In vitro and *in vivo* procedures were used to establish dosage. Blastogenesis was the *in vitro* test employed for establishing dosage in general. *In vitro* grown peripheral lymphocytes were challenged comparatively with the known mitogens phytohaemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) of varied concentrations. A dose responsive curve was established to evaluate the efficacy of ALF with that of the mitogens; 0.1 ml of 1:10 dilution of ALF was found to be optimal when administered twice weekly. Intradermal test was carried out on all recipients depending on their response and sensitivity. If unusual sensitivities were observed, the ALF was further diluted. This procedure was successively repeated until no adverse reaction was observed.

Results

Significant changes were observed within 1 to 6 weeks in patients treated with ALF. Changes were observed in immune regulation and overall clinical manifestations. With regard to clinical manifestations, there were noteworthy improvements (Table 1), although minimal symptoms continued after approximately 3 weeks of continued therapy.

In a normal cell cycle, the highest percentage of lymphocytes should be in the G_0 to G_1 phase (Figure 1). This percentage will change dramatically when these lymphocytes are stimulated by various incitants (Figures 3a and 3b). Consequently, different percentages will appear in the S and G_2M phases, producing a deregulated profile (Figures 1 to 4). Treatment with ALF regulates the T lymphocyte cell cycle profile.

Immunologically, there were significant regulations of T lymphocyte cell cycles, especially from one phase of the cycle to another. Patients became less sensitive and more tolerant to specific incitants. As treatment continued, in general in about 6 weeks, a more drastic shift toward that of a normal profile was observed. Figures 1 to 4 summarize the regulatory changes of some of the cell cycles studied.

Changes were observed in the profiles of the T and B cells where T and B lymphocytes and their subsets were evaluated before and after treatment. There was a significant ($p < 0.01$) change in the total lymphocyte count and subsets, T_4, T_8 in 92 patients investigated. It should be noted that ALF seems to act as an immune modifier since the total lymphocytes, T_4 and T_8 were significantly elevated or reduced in order to maintain normalization (Tables 2 and 3).

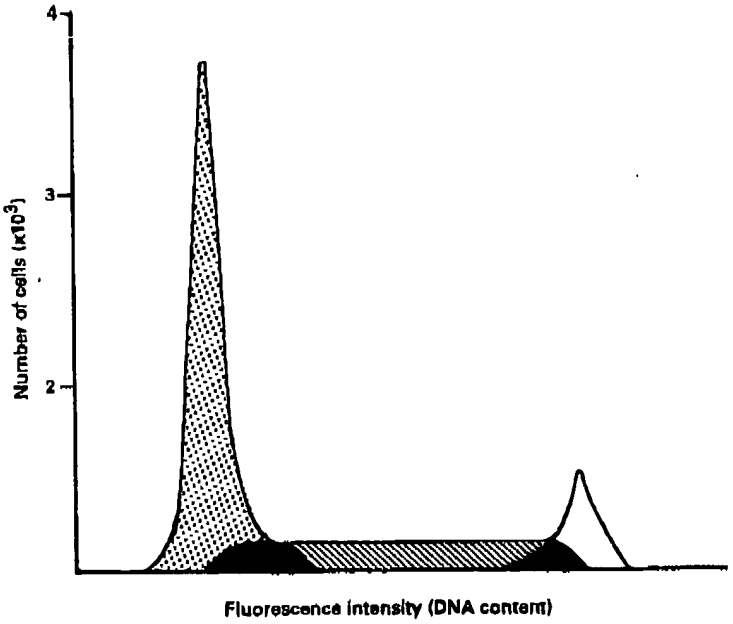

Figure 1 Diagrammatic representation of a normal mammalian cell cycle, showing the relative number of lymphocytes in each phase of the cycle, as displayed by the intensity of fluorescence of the fluorochrome-bound DNA in each lymphocyte. Normally, the percentage of lymphocytes in each phase of the cell cycle is 90–95, 5–10 and 0–5 in the G_0G_1 phase, S phase and G_2M phase, respectively. These quiescent lymphocytes display, normally, a low level of replication, and progression through the cell cycle. In response to stimuli, these cells undergo rapid division and cycle rapidly. The shadings show that there is overlapping of phases and not specific lines of demarcation: stippled, G_0G_1 phase (90–95% noncycling lymphocytes); hatched, S phase (5–10% cycling lymphocytes); unshaded (open), G_2M phase (0–5% cycling lymphocytes); and solid areas indicate overlapping of phases.

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Table 2 Representative changes in T and B cell profiles after 92 chemically sensitive patients were treated with autogenous lymphocytic factor

Treatment	Total lymphocytes	T_{11}	T_4	T_8	T_4/T_8	B_4
Before	2,112 ± 632	1,624 ± 457	930 ± 45	439 ± 58	2.3 ± 0.8	188 ± 102
After	2,232 ± 678*	1,634 ± 544	1,027 ± 297**	478 ± 189**	1.3 ± 7.4	171 ± 120

* $p < 0.05$; **$p < 0.01$; $n = 92$.

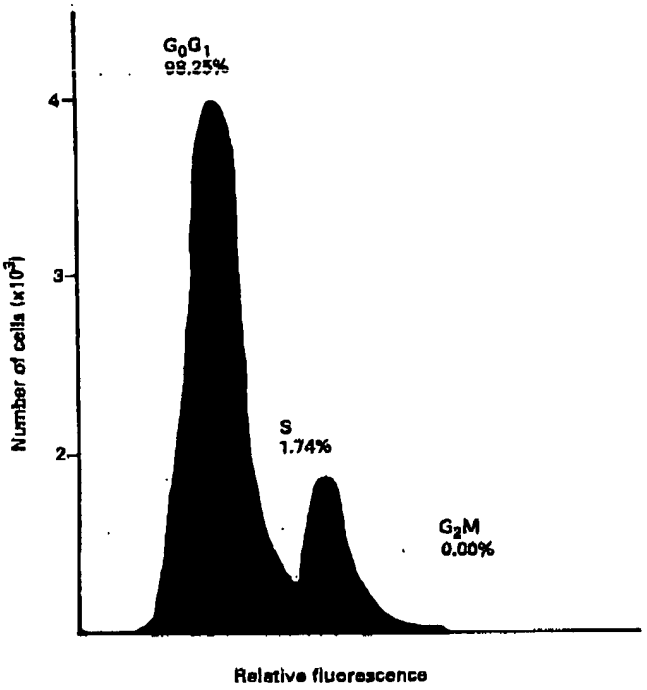

Figure 2 A representative DNA histogram of peripheral T lymphocytes from normal volunteers. Note that the highest percentage of lymphocytes is in the G_0G_1 phase, as expected, but the percentage varies in different individuals in keeping with the prevalence of environmental pollutants.[1] Thus, the ideal normal profile is seldom achieved. The abscissa shows the relative fluorescence of the fluorochrome-bound DNA in each cell.

Table 3 Profile of T lymphocyte subsets modulation after treatment with autogenous lymphocytic factor

After treatment	Total lymphocytes: Number	%	T_{11} Number	%	T_a Number	%	T_8 Number	%
Increase	52	57	41	46	53	53	55	60
Decrease	40	43	51	54	39	42	37	40
Probability	<0.05		>0.05		<0.05		<0.01	

Table 4a Typical cell-mediated response in (number and size) chemically sensitive individuals treated with autogenous lymphocytic factor

Patients	Before ALF	After ALF	P
190	5.46 ± 5.81	9.28 ± 7.25	<0.001

Results are given as mean ± SD.

Patients showed significant improvement ($p < 0.001$) in their CMI scores (Table 4). The regulatory effect of the immune system can be objectively assessed periodically after the initial treatment with ALF. The resourceful parameters are profiles of T lymphocyte cell cycle, T and B lymphocytes, and their subsets, cell-mediated immunity, signs and symptoms.

Side effects of ALF

The side effects were minimal and occurred only in six adults (five females and one male) where the average age was 52 years. These patients were intolerant of ALF, their symptoms included pain and irritation in the throat, burning in the eyes, pain and irritation in the chest, heat palpitations, influenza-like symptoms, headache, fatigue, and chills.

Table 4b Cell-mediated immunity positive score (number and size) after treatment with autogenous lymphocytic factor

Score	Patients	%	P
Increase	58	74	<0.001
No change	9	12	
Decrease	11	14	

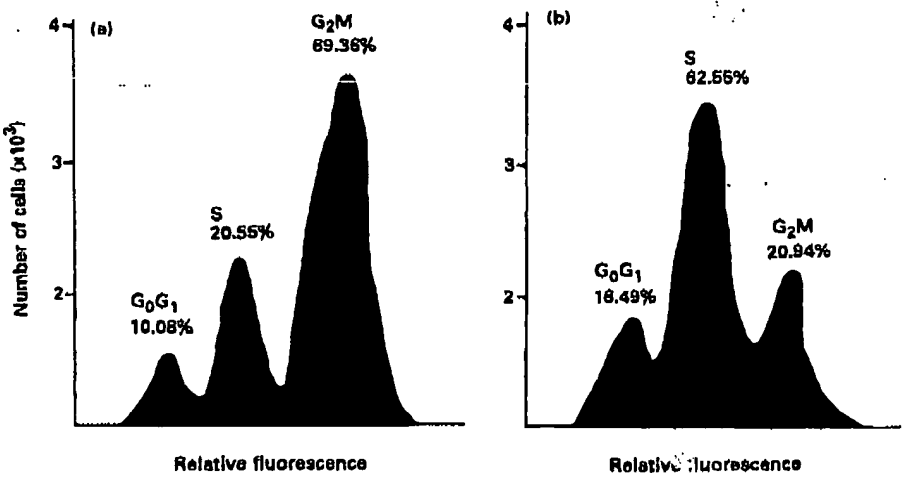

Figure 3a A representative irregular T lymphocyte cell cycle profile showing the effect(s) of stimulating the environmentally ill patients influence, primarily, the lymphocytes in the S phase.

Figure 3b A representative irregular T lymphocyte cell cycle profile showing the effect(s) of the stimulating incitants on the lymphocytes in the G_2M phase. It seems that each incitant, or a mixture of incitants affects lymphocytes in a particular phase(s) of the cell cycle, resulting in a variety of irregular cell cycle profiles and presumably dictates varied patterns of clinical manifestations. The abscissae in Figures 3a and 3b show the relative fluorescence of the fluorochrome-bound DNA in each cell.

Discussion

The flow cytometric profile of the T lymphocyte cell cycle as demonstrated by DNA histogram presents a reflection of the status of T lymphocytes in an individual. Of great importance is that its application is not limited to a certain category of individuals, but to normal subjects as well as individuals who are compromised by varied incitants. The essence of its clinico-biological importance, as detected in the present investigation, is that it is a reflection of T lymphocyte cell function, and may facilitate an in-depth approach to the treatment of some immunodeficient illnesses.

The progression of T lymphocytes from one phase of the cell cycle to another is time-dependent, ~8 to 12 h in the G_0-G_1 phase, 6 to 8 h in the S phase, and 0.5 to 1 h in the G_2M phase. The 290 individuals who were investigated in this study were affected principally by environmental incitants in categories such as foods, biological inhalants, and chemicals. They presented histories of varied backgrounds and different cell cycle profiles. The DNA histogram

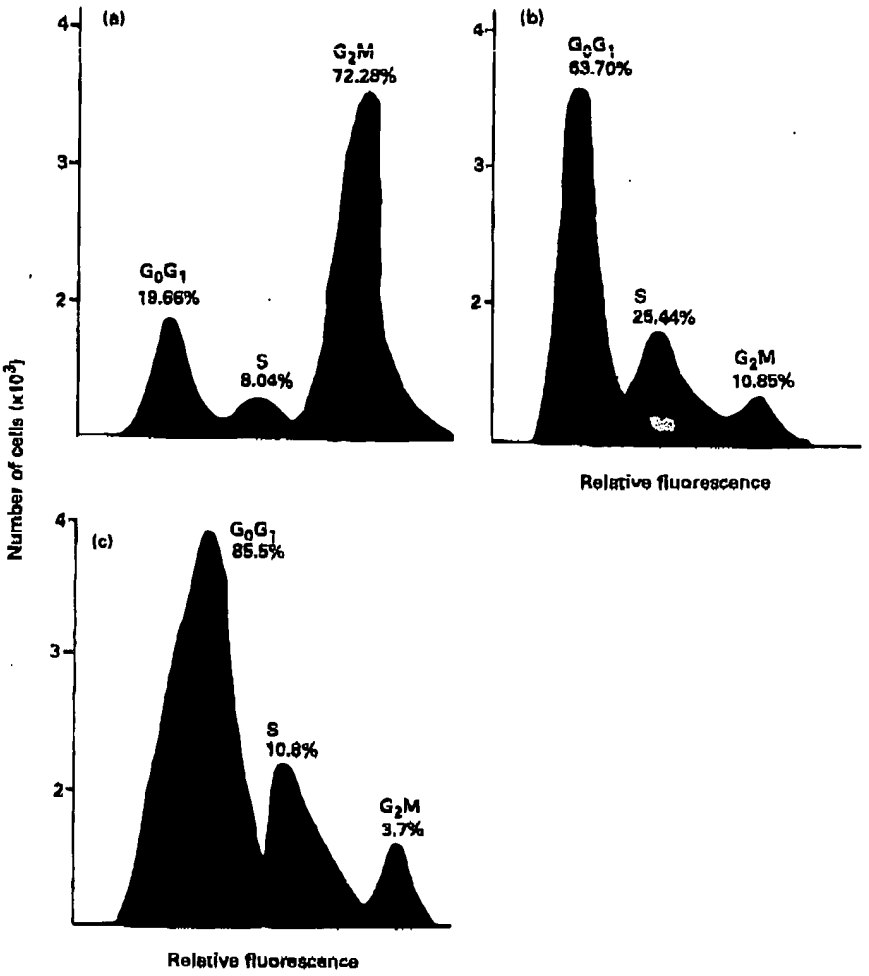

Figure 4a Initial T lymphocyte cell cycle profile of a patient on examination at the clinic. Note that the irregularity of the profile is emphasized in the G_2M phase.

Figure 4b Cell cycle of the patient in Figure 4a after the subject was treated with autogenous lymphocytic factor (ALF) for 3 weeks. Note that the percentages of lymphocytes in each phase of the cell cycle are still irregular, but there has been a significant improvement. The highest percentage of lymphocytes now appears in the G_0G_1 phase.

Figure 4c T lymphocyte cell cycle of patient (Figures 4a and b) subsequent to 3 months treatment with ALF. Note that the cell cycle has attained a relatively normal profile. ALF seems to act as a biological response modifier. The abscissae in Figures 4a, b and c show the relative fluorescence of the fluorochrome-bound DNA in each cell.

cycles showed over or under accumulation of lymphocytes in one or more phases of the cell cycle of each individual (Figures 3a and 3b). As shown in Figure 2, even the normal controls do not present an ideal DNA histogram profile of T lymphocytes. This is due to the fact that, in general, the ideal environment is seldom ever achieved, thus there is always some degree of immunological compromise to most individuals. It is reasonable then to assume that the incitant(s) which lead to immuno-incompetence of these individuals are capable of inactivating the particular enzyme(s), cyclin-dependent kinases, or depleting specific cyclins whose combination with their specific kinases are instrumental in catalysing the progression of T lymphocytes from one phase to another of the cell cycle. The T lymphocytes of the individual affected are locked in a particular phase, either resting, synthesizing, or multiplying too much in the G_0-G_1, S, G_2M phase respectively. Thus the subject manifests symptoms peculiar to the phases(s) affected. This hypothesis offers an opportunity to associate clinical manifestations with T lymphocyte cell cycle irregularity.

The treatment of lasting importance would be reasonably thought of as a biological response modifier, which would stimulate CDKs, regulate the cell cycle and the enzymes of purine and pyrimidine nucleotide synthesis. It is now generally accepted that these enzymes are elevated during the S phase (Cory, 1993). It seems logical that ALF stimulates these regulatory functions. The autogenous lymphocytic factor is a dialysable mixture of the many effector substances which may be released from *in vitro* grown stimulated lymphocytes with the ability to invoke immunological influences *in vivo* or *in vitro*, (Griffiths and Rea, 1995). The expected biological activity(s) of ALF would be to act as a biological response modifier, with mechanisms of action as suppressor and regulator of the immune system, especially in the regulation of the T lymphocyte cell cycle.

Kamp *et al.* (1994) showed that progression through the cell cycle requires the joint influence of positive regulator subunits, cyclins, and cyclin dependent kinases. These subunits regulate by phosphorylation a number of key substrates which subsequently activate a transition from G_1 to S, and G_2 to M phases of the cell cycle. Phosphate groups are transferred from ATP to a special amino acid in the target protein by protein kinases, while phosphatases remove the phosphate groups from the target proteins. The addition and removal of phosphate groups significantly affect the biochemical behaviour of the target proteins. Many protein kinases and phosphatases have a specific affinity for their target proteins, and act as determinants for controlling the activity(s) of their target

proteins. Indeed, ALF may possess these protein kinases which, acting as molecular switches, regulate an irregular T lymphocyte cell cycle to that of an ordered and orderly progression.

As demonstrated (Figure 4c), ALF regulates deregulated cell cycles, improves immunological profiles by increasing or decreasing the number of circulating lymphocytes and their subpopulations (Tables 2 and 3); and restores immune responses as demonstrated by enhanced cutaneous hypersensitivity (CMI) (Table 4). ALF also invoked immediate intradermal test response within 45 min of administration subsequent to the administration of antigen challenges which were previously negative or delayed for at least 15 days. Responses were observed by the number and size of wheals to the antigens which were inoculated. It is noteworthy that some chemically sensitive patients responded favourably, or completely recovered after environmental exposure to air, food, water, nutritional support and exercise. Some patients responded but did not fully recover. The patients who recovered reacted profoundly to exposure of very minute environmental insults of ambient chemicals, biological inhalants, and foods, or inhalants. However, when these patients were treated with ALF, their hypersensitivity was markedly reduced or disappeared; there was also significant improvement in recurrent infection, fatigue, headaches, depression, concentration, and gastrointestinal upsets. Eight patients immediately improved on initial injection with ALF.

T-suppressor, T-helper cells, and total lymphocytes were increased as reflected in an increase in a low population, and a decrease in a too high population. This suggests that ALF is a modulator rather than a stimulator. The mechanism(s) of action of ALF have not yet been ascertained. Indeed, the purification and determination of ALF from sorted T lymphocytes with or without helper phenotypes will be necessary to facilitate understanding the mechanism of action. However, it has been documented that the human cells contain a regulatory protein, CKS protein, which is a genetic suppressor of temperature sensitive CDK mutations. There are two isoforms of this CKS protein, namely CKSH1 and CKSH2. It is believed that CKSH2 protein binds to the catalytic subunit of the CDKs, and is essential for their biological function (Parge *et al.*, 1993). To this end, further investigations are being carried out.

The concept of a functional cell cycle is timely. Indeed, the profile of a cell cycle offers an insight into the immune status of a patient. However, the cell cycle profile does not indicate whether or not the lymphocytes are immunologically responsive or capable of responding to therapy. The influence of varied environmental incitants and/or

other systemic illnesses may render these lymphocytes incapable of normal progression through the cell cycle, thus resulting in a non-functional cell cycle. *In vitro* lymphocyte activation represents a standard procedure for evaluating cell mediated responses to a variety of stimuli including antibodies, polyclonal mitogens, specific antigens and cytokines. Interleukin-1 alpha (Il_1 alpha) and ALF were used as activators in the present investigation. Interleukin-1 alpha was used due to its extremely broad spectrum of bioactivity. ALF was used to observe its capability as a biological response modifier. The stimulatory process presented in this investigation offers a method of computing lymphocyte stimulatory response, and consequently an opportunity to establish an individual regime of treatment of illnesses associated with immunodeficiency. This investigation offers: (1) a vivid picture of the status of the immune profile of a patient as demonstrated by the DNA histogram of the T lymphocyte cell cycle; (2) an opportunity to regulate an irregular T lymphocyte cell cycle by treatment with autogenous lymphocytic factor; and (3) a clinical tool for the regulation of abnormal cell cycles and/or haematological and immunological profiles in humans with immunological deregulation. It should be noted that there is a pending United States patent, number 08/380,063 for the application of the cell cycle to clinical medicine, and also the regulation of the cycle by autogenous lymphocytic factor.

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